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Self renewal & pluripotency

1.

Modulating stem cell properties by transducible Oct4 and Sox2 transcription factors

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Keywords: Oct4, pluripotency, Sox2

Stem cells have the unique capacities to self-renew and give rise to differentiated cell types. Gaining control over the mechanisms of stemness may provide a means to promote unlimited proliferation and/or dedifferentiation. Oct4 and Sox2 have been identified as key regulators of pluripotency. Together with the homeodomain protein Nanog these factors shape a core transcriptional network that controls two types of target genes with opposing functions: pluripotency genes and differentiation genes.

In order to analyze this stemness transcriptional network we aimed to generate cell permeable versions of these factors, which can be used for direct cellular manipulation employing cell-permeant biologically active proteins. This method enables application of transcription factors in a controlled manner without genetic modification of the target cells.

For that we tagged proteins with a protein transduction domain (TAT-sequence), which enables them to pass the cell membrane. In this study we engineered different versions of the transducible factors to identify the optimal candidates for recombinant expression and purification of the proteins. The purified recombinant proteins are able to bind specifically their corresponding DNA target sequences indicating functionality of the proteins. The path of the recombinant proteins into cultured cells was visualized by rhodamine labeling. Addition of the TAT-sequence to the protein enhanced its cellular uptake. We observed that transduction of each factor Oct4 and Sox2 resulted in improved stem cell properties as judged by the morphology of ES-cell colonies.

2.

Reprogramming potential of murine parthenogenetic embryonic stem cells

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Keywords: embryonic stem cells, parthenogenesis, reprogramming

Background & aims
Mammalian oocytes can be induced to cleave if extrusion of the first polar body is inhibited or reverted. The process, named parthenogenetic activation, can give rise to blastocysts. The inner cell mass of blastocysts can be isolated and used to derive parthenogenetic embryonic stem cell lines (pES). In our present study we investigated whether pES by cell fusion can reprogram somatic cells to acquire a pluripotent state.

Methods & results
As a somatic fusion partner we chose neural stem cells (NSC) that carry two transgenes, Oct4-GFP and Rosa26-Neo. After fusion of these cells with pES cells, colonies appeared that were GFP-positive and neomycin-resistant. The presence of such colonies showed reactivation of Oct4 expression, suggesting reprogramming of NSCs. The fused cells were further investigated by RT-PCR and immunofluorescence for the presence of Oct4 and Nanog. Ploidy analyzes confirmed the tetraploid state of the pES–NSC. After transplantation into SCID-mice pES as well as pES/NSC gave rise to teratomas, comprising cells from all three germ layers. In addition, several tests, such as DNA-methylation, were performed to demonstrate that reprogramming in the pES–NSC fusion hybrids had occurred.

Conclusion
The pES has the reprogramming potential to convert somatic cells into a pluripotent state. Therefore, pES might be an alternative source for reprogramming strategies such as fusion with somatic cells as well as for the isolation of reprogramming factors from cell extracts.

3.

Significance of FGF2 signaling in human embryonic stem cells and human embryonal carcinoma cells in the context of maintaining the undifferentiated state

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Keywords: differentiation, FGF2, gene regulation, human embryonic stem cells, self-renewal, TGFß

Self-renewal of human embryonic stem cells (hES cells) requires both FGF2 signaling and activation of SMAD 2/3 via the Activin/Nodal/TGFβ branch of the TGFβ pathway (Amit *et al.*, 2000, James *et al.*, 2005). It has been shown that the beneficial effect of FGF2 is dependent on TGFβ receptor function (Vallier *et al.*, 2005). Xu *et al.* (2001) have developed a widely used protocol for feeder-free growth of hES cells, which involves media supplementation with FGF2 both before and after the MEF conditioning step. We have re-investigated the physiological rationale for this and show that FGF2 is a modulator of TGFβ signaling both in MEFs and hES cells, supporting hES cell self-renewal via activation and repression of key TGFβ ligands.

Using human embryonal carcinoma (hEC) cells as an easy-to-manipulate model to study self-renewal in human pluripotent cells, we performed a medium-scale RNAi screen targeting common hES/hEC marker genes, followed by whole-genome expression analysis. These revealed overall downstream targets of core transcription factors as well as a critical role of autocrine FGF signaling for self-renewal of hEC cells, as in hES cells.

4.

Chromosome localization and expression of the pluripotency markers in the embryonic stem cells of American mink (mustela vison)

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Keywords: American mink embryonic stem cell, pluripotency markers

Embryonic stem (ES) cells are a valuable system to study early development of mammals. A lot of researchers are dedicated to comprehensive study of the ES cells. Today ES cells have been isolated from mice, human and some other mammalian species. A set of the American mink ES cell lines had been isolated from mink morulas and blastocysts in our group. The formation of embryoid bodies and teratomas in athymic mice suggested that these cells are pluripotent.

It is known that several transcription factors are essential for the ES cell identity, including two homeodomain proteins, Nanog and Oct-4, and a HMG factor, Sox-2. These factors influence on other transcription factors and control expression of each other establishing regulatory network that maintains ES cell pluripotency and self-renewal. In this study, we analyzed the expression of the Oct-4 and Sox-2 genes in the American mink ES cells and determined their chromosomal localization.

First we determined the primary structure of the American mink Oct-4 and Sox-2 gene fragments. Using the Chinese hamster–American mink somatic cell hybrids panel, consisting of 14 clones, we localized Oct-4 and Sox-2 genes to mink chromosomes one and six, respectively, with 100% concordance between the marker and the specific chromosome.

To analyze the expression of Oct-4 in the mink ES cell lines (MES-12, MES-12neo and MES-13) we used RT-PCR analysis and immunostaining. Both methods revealed that the ES cells expressed Oct-4. As for Sox-2 with use of RT-PCR we have found the low level of expression in the ES cells. The expression of pluripotency markers confirms the pluripotent capacities of examined ES cells.

Disclosures
The study was supported by Russian Academy of Sciences presidium grant for Fundamental Researches (11.7).

5.

Transcription factor repertoire of mouse Flt3$^+$ multipotent hematopoietic stem/progenitor cells and embryonic stem cells

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Keywords: embryonic stem cells, gene expression profiling, hematopoietic stem cells, transcription factors

Hematopoietic stem cells (HSCs) maintain the development of all mature blood cells throughout life due to their sustained self-renewal capacity and multilineage differentiation potential.

In mammals the HSC compartment contains long-term reconstituting cells (LT-HSC) that generate all hematopoietic lineages for life, and short-term HSC (ST-HSC), which are multipotent progenitors with limited self-renewal capacity. The fms-like tyrosine kinase-3 ligand (Flt3L) has emerged as a potent growth factor for stem/progenitor cells expressing Flt3 receptor, and bone marrow from Flt3$^{-/-}$ mice display reduced reconstitution activity *in vivo*. In mouse, LT-HSC are Lin$^-$Sca-1$^+$c-kit$^+$ and Flt3$^-$ whereas ST-HSC are Lin$^-$Sca-1$^+$c-kit$^+$ and Flt3$^+$, and this associates Flt3 expression with loss of sustained self-renewal potential.

In addition to hematopoietic cytokines and growth factors, stem cell renewal and cell fate choice in hematopoiesis have been proposed to be regulated by instructive signals provided by the microenvironment. This includes, for example, cell–cell contacts, Wnt and Notch factors that coordinate activities of multiple signaling pathways. However, all extrinsically activated signaling pathways within stem/progenitor cells will integrate in orchestrated gene expression programs driven by specific transcriptional regulators.

We developed a culture system for amplifying a multipotent $Flt3^+$ progenitor from mouse bone marrow using a specific cytokine/growth factor combination that includes Flt3L. This culture system yields homogeneous populations of $Flt3^+CD11b^+$ progenitor cells in high numbers. Here we report on the developmental potential of $Flt3^+CD11b^+$ progenitor cells into various mature blood cells *in vivo* using bone marrow transplantation. We studied phenotypic and functional properties of $Flt3^+CD11b^+$ progenitor cells and present a comprehensive analysis of their transcription factor repertoire determined by gene expression profiling with DNA microarrays. We compared the transcription factor expression of $Flt3^+CD11b^+$ progenitors, highly purified $Lin^-Sca\text{-}1^+c\text{-}kit^+$ HSC and embryonic stem cells, and defined overlapping and nonoverlapping transcription factor repertoires. This study provides novel insights into the dynamic networks of transcriptional regulators in embryonic and adult stem cells, and thus opens the perspective for elucidating lineage and 'stemness' determinants in hematopoiesis.

Reference

1. Hieronymus T, Gust TC, Kirsch RD *et al.*: Progressive and controlled development of mouse dendritic cells from $Flt3^+CD11b^+$ progenitors *in vitro*. *J. Immunol.* 174(5), 2552–2562 (2002).

6.

Novel nonhuman primate embryonic stem cells from marmosets (*Callithrix jacchus*): influence of feeder cell treatment on embryonic stem cell growth

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Keywords: embryonic stem cells, feeder cells, MEF, nonhuman primate model, self-renewal

Embryonic stem cells (ESCs) hold tremendous potential for therapeutic applications, including regenerative medicine, as well as for understanding basic mechanisms in stem cell biology. Many experiments cannot be conducted in human ESC because of ethical or practical limitations and thus nonhuman primate ESCs serve as invaluable clinically relevant models. The novel marmoset (Callithrix jacchus) ESC line CJES001 was characterized using different stem cell markers. The cells stained positively with Oct3/4, SSEA-3, SSEA-4, Tra-1-60, Tra-1-81 and Sox-2, underscoring their status as undifferentiated ESCs. ESCs are typically grown on mouse embryonic fibroblasts (MEF) as feeder cells whose proliferation is arrested either by treatment with Mitomycin C or by γ-irradiation. To assess the impact of these treatments on the ability of MEF to support growth of undifferentiated ESC, we quantified 69 cytokines and growth factors in the supernatant of both Mitomycin-treated and γ-irradiated MEF by bead-based multiplex analysis and thus established a profile of MEF-secreted factors. The time course of secretion was analyzed by monitoring the supernatant at 0, 6, 12, 24 and 36 h. Comparing γ-irradiated and Mitomycin-treated MEF suggested higher amounts of some cytokines including IP10, Insulin and Eotaxin by the former. We also assessed whether the method of inactivation had an effect on growth kinetics and differentiation of primate ESCs. First, we used an MTT assay to evaluate the cellular metabolic activity of growth-arrested feeder cells. There was a significant ($p < 0.02$) difference between the different ways of inactivation with γ-irradiated cells displaying a higher metabolic activity. However, this difference was not reflected in the kinetics of ESC growth and the morphology of undifferentiated ESC colonies. There appeared to be a trend to a lower number of differentiated ESC colonies on the γ-irradiated feeder cells, suggesting that this may be a preferable method of growth arrest.

7.

Novel protocol for maintaining human embryonic stem cells pluripotency in prolonged maintenance culture

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Keywords: alginate, cell aggregates, encapsulation, human embryonic stem cells, pluripotency

Most current means to maintain human embryonic stem cells (hESCs) pluripotency require support from human or animal feeder cell layers, the most common being murine embryonic fibroblasts. In this study, we applied a protocol aimed at maintaining hESCs in culture without exposure to animal cells or proteins. The hESC colonies were encapsulated in 1.1% alginate hydrogel and grown in basic maintenance medium for periods up to 260 days. Investigation of the cell aggregates after this prolonged culture yielded no evidence of the formation of any of the three germ layers, although the cells could differentiate normally when they were subsequently cultured in a conditioned environment. Immunohistochemistry and RT-PCR showed that the hESC aggregates expressed protein and gene markers of pluripotency including Oct-4, Nanog, SSEA 4, TRA-1-60 and TRA-1-81. At the ultrastructural level, the cells were arranged in closely packed clusters and showed no cytoplasmic organelles, suggesting an undifferentiated state. These data show that it is possible to maintain hESCs in an undifferentiated state, without passaging or embryoid body formation, without animal contamination. Our findings represent a significant step towards providing completely animal-free conditions for future therapeutic applications of hESC biology.

8.

Downstream targets of the embryonic specific transcription factor, OCT4: the gatekeeper of pluripotency

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Keywords: NANOG, NCCIT, OCT4, SOX2

The transcription factor OCT4, is one of the key regulators responsible for maintaining pluripotency in undifferentiated stem cells. It is highly expressed in unfertilized oocytes, primordial germ cells, the inner cell mass of the blastocyst, embryonic stem (ES) and EC cells. Ablation of its function in both ES and EC cells results in morphologic and transcriptional changes, reflecting the early differentiation processes these cells go through. EC cells are stem cells derived from teratocarcinomas, and are the malignant counterparts of ES cells derived from the inner cell mass of blastocyst-stage embryos. Similar to ES cells, they are pluripotent, which means that they can differentiate into any derivative of the three primary embryonic germ layers. OCT4 together with SOX2 and NANOG form the core transcriptional regulatory circuitry in human ES cells. The best described binding sites so far for OCT4 are located within the promoters of SOX2 and NANOG. Employing the chromatin-immunoprecipitation technique, we have demonstrated an enhanced enrichment for these binding sites compared with other unrelated promoter regions such as the hemoglobin β chain. We chose to hybridize three biological replicates from NCCIT cells onto a NimbleGen™ promoter tiling array, containing 5.0 kb of promoter region for a comprehensive set of genes. All annotated splice variants and alternative splice sites are represented. Where the individual 5.0 kb regions overlap, they are merged into a single larger region, preventing redundancy of coverage. The promoter regions thus range in size from 5.0 to 35 to 50 kb. These regions are tiled at a 110 bp interval, using variable length probes with a target Tm of 76Â°C. We have defined a peak finding algorithm and checked different threshold levels (from 2.0 to 1.5 in at least two replicates).This high resolution resulted in average peak sizes of around 500 bp. Based on our definition our most stringent dataset (all three replicates must have a threshold above two for at least three probes in a given window) resulted in 100 direct potential targets for OCT4. Of these, 83% contained the classical SOX2:OCT4 motif, which has been described before. Using the PWM of this motif based on our data, we screened the lesser stringent datasets for the presence of SOX2:OCT4 motifs, OCT4 motifs and NANOG

motifs, resulting in 465 targets with OCT4-related motifs and 126 targets with NANOG related motifs. Furthermore, we have identified a subset of genes bearing the OCT4 motif with yet to be characterized binding motifs adjacent to the OCT4 binding site. These have been confirmed using EMSA. We are now assigning conservation scores for our target genes to sort out highly conserved OCT4-bound genes that will be functionally characterized in the context of maintenance of self-renewal in ES cells.

9.

Phenotype of hybrid cells generated by fusion of embryonic stem cells with tetraploid fibroblasts

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Keywords: embryonic stem cell, hybrid cells, segregation

Hybrid cells generated by fusion of pluripotent embryonic stem (ES) cells and somatic cells are a good experimental model for the study of pluripotency and somatic nucleus reprogramming. It is known that hybrid cells generated by fusion of diploid ES and somatic cells have a phenotype of pluripotent partner. The aim of this work is to investigate whether a ploidy of somatic partner affects the phenotype of hybrid cells.

We used hybrid cells produced by fusion of ES cells (E14Tg2aSc4TP6.3 line) with diploid and tetraploid embryonic fibroblasts obtained from two mouse cell lines DD/c and C57Bl/6-I (I) 1RK. We generated 12 ES–diploid fibroblasts hybrid cell clones and 12 ES–tetraploid fibroblast hybrid cell clones (C57Bl/6-I(I)1RK line); 18 ES–diploid fibroblast hybrid cell clones and 12 ES–tetraploid fibroblast hybrid cell clones (DD/c line). It was found that all ES-tetraploid fibroblast hybrid cells had a fibroblast-like phenotype. It was shown by immunofluorescent analysis that the presence of type I collagen and pattern of f-actin in ES–tetraploid fibroblast hybrid cell cytoplasm are the same as in fibroblast cells. By contrast, all hybrid cells generated by fusion of ES cells with diploid fibroblasts had a phenotype of pluripotent partner. Therefore, a percent age of cells with Oct-4 and Nanog expression in such hybrid clones is the same as in the parental ES cells.

It could be assumed that chromosome segregation can cause somatic or pluripotent phenotype domination in hybrid cells. PCR analysis of polymorphic microsatellites allowed us to mark chromosomes of both partners. Considerable segregation of the pluripotent partner chromosomes was found in ES–tetraploid fibroblast hybrid cells. PCR analysis of ES–diploid fibroblast (DD/c line) hybrid cells have not shown chromosome segregation and only few chromosomes of somatic partner segregate in some ES–diploid fibroblast hybrid clones (C57Bl/6-I(I)1RK line). It could be expected that hybrid cells obtained by fusion of two diploid cells would be a tetraploid (80 chromosomes). Cytogenetic analysis data showed the presence of 74–76, and in some clones 66–68, chromosomes. Probably, segregation of several homologs of parental chromosomes occurred in all ES–diploid fibroblast hybrid clones. Thus, we firstly described a somatic partner ploidy affect to phenotype and pluripotent partner chromosome segregation in ES-tetraploid fibroblast hybrid cells.

Disclosure
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10.

NUMB, a cell fate determinant known from *Drosophila melanogaster*, inhibits maintenance of primitive human hematopoietic cell fates

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Hematopoietic stem cells are the most investigated mammalian stem cells. Like other stem cells they are undifferentiated cells that can self-renew over a long period of time and give rise to progenitor cells that will reconstitute the whole immune and blood system. Although the mechanisms regulating the decision process self-renewal versus differentiation remain largely unknown, there is good evidence that a combination of both extrinsic and intrinsic factors as well as the Notch signaling pathway are involved in controlling the cell fates of primitive hematopoietic cells and their arising daughter cells.

Similarly, the Notch signaling pathway, its extrinsic ligands and its intrinsic modulators specify the cell fates of the four cells of developing external sensory organs of Drosophila melanogaster. In this system, the so-called sensory organ precursor cells divide asymmetrically to give rise to IIa and IIb daughter cells. Mechanistically, the cell fate determinant Numb, an antagonistic protein of Notch, segregates mainly into IIa daughter cells and inhibits the signal transduction of Notch, being activated by its ligands Delta and Serrate (the homolog of mammalian Jagged). Depending on the transduction of the Notch signal, daughter cells are either specified as IIa (no transduced signal) or as IIb (transduced signal).

Since Numb is conserved during evolution and Notch activity seems to be required to maintain primitive hematopoietic cell fates, we wondered whether Numb is involved in cell fate specification during early hematopoiesis as well. GeneChip analyzes performed in collaboration with N Ivanova and I Lemischka (Princeton University) or with L Klein-Hitpass, T Moritz and J Thomale (Universitätklinikum Essen) together with numb specific RT-PCR analyzes revealed that numb is expressed in primitive hematopoietic cells of the $lin^-CD34^+CD38^{low/-}$ and $lin^-CD34^+CD38^+$ cell fractions. By immunohistochemical and flow cytometric analyzes we could further show that even on the protein level Numb is expressed in all $CD34^+$ cells. To functionally analyze the impact of Numb on the biology of primitive hematopoietic cells, in other words on $CD34^+$ cells, we performed over-expression experiments and realized that enforced Numb expression inhibits the maintenance of primitive hematopoietic cell fates. In contrast to this and in agreement with published data, $CD34^+$ cells expressing a constitutive active variant of Notch-1 contained a higher rate of more primitive cells than $CD34^+$ cells of corresponding controls.

11.

Cell permeable Nanog protein increases proliferation and suppresses cellular senescence of somatic cells

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Keywords: Nanog, pluripotency, protein transduction, self-renewal, senescence

Embryonic stem cells differ from somatic cells by their unique capacity to renew themselves and to give rise to all differentiated cell types. The transcription factors Oct4, Nanog and Sox2 build a core transcriptional network that constitutes embryonic stem cell identity. These three factors co-occupy the promoters of a large population of genes encoding developmentally important homeodomain transcription factors. Moreover, Nanog alone promotes transfer of pluripotency to somatic cells fused to embryonic stem cells. We present a novel experimental strategy to induce Nanog activity into cells in a protein-based manner thereby reversibly modulating stem cell properties without genetic modification. Based on our previously reported Cre protein transduction system we engineered a cell permeable version of the stem cell factor Nanog (TAT–Nanog fusion protein). Treatment with TAT–Nanog induces self-renewal properties in embryonic stem cells even in the absence of leukemia inhibitory factor (LIF) in a reversible manner. Moreover, we show the potential of TAT–Nanog to modulate growth properties of mature cells. For that we used NIH 3T3 cells and murine embryonic fibroblasts as model systems. Introduction of Nanog activity into somatic NIH 3T3 cells resulted in an increased growth rate and a transformed phenotype as demonstrated by foci formation and colony growth in soft agar. TAT–Nanog transduction into primary MEFs induced an increased proliferation and bypassed senescence resulting in an ongoing cell expansion. In conclusion, our system of cellular manipulation provides a powerful model to introduce stemness factors into somatic cells without altering the genome and overcoming gene silencing. We expect this approach to greatly facilitate the analysis of the molecular link between stemness, epigenetic modifications and transformation.

12.

Characterization of neural stem cells isolated from Tlx mutant mice

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Keywords: clonal analysis, epidermal growth factor receptor, FACS, neural stem cells, Tlx

Neural stem cells (NSC) are present both in the embryonic and adult brain. After birth neurogenesis continues in the subventricular zone (SVZ) of the lateral ventricle and in the dentate gyrus of the hippocampus, generating, respectively, new olfactory bulb interneurons and granule neurons. Adult mice lacking the expression of orphan nuclear receptor tailless (tlx) show a severe impairment of adult neurogenesis and precursor proliferation in both neurogenic regions. Previous evidence suggests that Tlx is an intrinsic factor involved in the proliferation and self-renewal of adult NSCs preventing their differentiation into astrocytes. However, it is not clear whether Tlx regulates NSC proliferation only in the adult brain or also earlier in development. Here we have taken advantage of a NSC isolation procedure to directly characterize NSCs derived from different brain regions of $Tlx^{-/-}$ and WT mice at various developmental stages.

Methods

To isolate NSC we have used epidermal growth factor (EGF) tagged with a fluorescent fluorophore and flow cytometry to detect levels of EGF receptor (EGFR) in dissociated neural cells. Using this approach we previously isolated small cell populations displaying high levels of EGFR (EGFRhigh cells) from different regions of the embryonic telencephalon and the adult SVZ. We showed that at least one in five EGFRhigh cells displays *in vitro* stem cell activity such as clone formation, long term-self-renewal and multipotentiality. We have also showed that EGFR high cells derive from NSCs displaying low levels of EGFR

(EGFRlow cells) and that exposure to fibroblast growth factor (FGF)-2 promotes upregulation of EGFR levels in a subset of EGFRlow NSCs. Using this approach we have analyzed SVZ NSCs isolated from WT and $Tlx^{-/-}$ mice across development. We have also used immunohistochemistry to determine expression of differentiation stage markers in the SVZ of WT and $Tlx^{-/-}$ animals. Finally to better understand the function of Tlx in NSCs we have overexpressed Tlx in WT striatal cells.

Findings/conclusion

Firstly, we have analyzed NSCs from the embryonic (E18) striatum. At this stage, irrespective of EGFR levels we found no difference in clone formation between WT and $Tlx^{-/-}$ mice. However the population of EGFRlow cells that upregulated EGFR levels upon stimulation with FGF-2 was significantly decreased in mutant animals. Furthermore, overexpression of Tlx in embryonic striatal WT cells led to an increase in the number of EGFRhigh clone-forming cells.

Similarly at postnatal day 7 (p7), the number of SVZ EGFRhigh NSCs and their proliferative ability were not significantly affected by Tlx absence. However, compared with WT, in mutant mice a smaller number EGFRlow cells increased EGFR expression in response to FGF-2. Furthermore, at this age we also found a severe reduction in the number of EGFRlow NSCs. At this age immunohistochemistry also revealed a drastic increase of GFAP expression in the periventricular area of $Tlx^{-/-}$ mice. Finally, in adult $Tlx^{-/-}$ mice the number of both EGFRhigh and EGFRlow NSCs was dramatically reduced compared with WT.

Taken together, the effect of Tlx on NSC proliferation is not stereotypical but depends on the developmental stage. At earlier developmental stages absence of Tlx mostly affects proliferation of EGFRlow NSCs. This effect is paralleled by an increase in GFAP expression in the germinal zone surrounding the lateral ventricle. By contrast, later in development absence of Tlx leads to a depletion of both EGFRhigh and EGFRlow NSCs.

13.

Protein tyrosine phosphatases are spatially and temporally regulated in the developing retina and are expressed in the retinal stem cell niche

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Keywords: retinal progenitor/stem cells, retinospheres, protein tyrosine phosphatases

Protein tyrosine phosphatases (PTPases) appear to coordinate many aspects of neural development, including cell proliferation, migration and differentiation as well as axonal growth and guidance. Recent studies demonstrated that various receptor- and intracellular PTPases are differentially expressed in the developing mouse retina (Horvat-Bröcker *et al.*, in revision 2007, Klausmeyer *et al.*, in press 2007).

In this study we focus on the intracellular PTPase MEG2 and the receptor protein tyrosine phosphatase RPTP-β to get further insight concerning the influence of these candidates on retinal development.

The spatial and temporal expression of MEG2 and RPTP-β was analyzed in detail using quantitative real-time PCR, immunhistochemistry and immuncytochemistry. To elucidate the potential functional role of both PTPases in retinal progenitor/stem cells we established an *in vitro* culture system in mice, called 'retinospheres'.

The PTPase MEG2 appeared down-regulated in late embryonic as well as in postnatal stages, when compared with E13. We present here the first evidence that at E13 MEG2 is strongly expressed by proliferative retinal progenitor cells and in postmitotic newborn retinal neurons. Postnatally, at P20, MEG2 was detectable in ganglion, amacrine, bipolar and horizontal cells as well as in the outer segments of photoreceptors. The most prominent expression was observed in the upper part of the inner plexiform layer. Furthermore, we could demonstrate the *in vitro* expression of MEG2 in differentiated retinospheres at E18. The expression of MEG2 is mainly restricted to nestin, Pax6 and β-III-Tubulin immunoreactive cells.

Immunhistochemical analysis of RPTP-β demonstrated its expression in actively cycling cells in the outer neuroblast layer. Additionally, it could be shown that at early embryonic stages Pax6- and Nestin-expressing progenitor cells are immunpositive for the RPTP-β isoforms. As development proceeds a switch occurs, in that RPTP-expressing cells convert from a progenitor status to a more differentiated state partially positive for β-Tubulin, NeuN, BLBP and Recoverin. Immuncytochemical analysis of acutely dissociated retinal cells from E13.5 to E18.5 revealed that 15–17% express the 473HD-epitope. Approxiamtely 30% of these cells actively proliferate. At E13.5 half of DSD-1-epitope bearing cells are nestin-positive progenitors. This number

decreases at E15.5 and increases at E18.5. Vice versa, the cell population co-expressing Tubulin increases at E15.5 and decreases at E18.5. Nevertheless, we could also detect a small population of more differentiated cells expressing RPTP-β, dependent on the developmental state of the retina.

Taken together, these observations suggest that the PTPases MEG2 and RPTP-β play an essential role in developmental processes such as retinal progenitor/stem cell proliferation and differentiation. Knockdown experiments using shRNA *in vitro* and *in vivo* will generate further insight concerning the influence of both candidates on retinal development.

14.

Population of neural stem/progenitor cells appears in non-neurogenic cortical areas as a consequence of defined acute laser lesions

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Keywords: adult stem cells, endogenous progenitors, focal laser lesion, multipotency, proliferation

Several studies in the last few years have confirmed the occurrence of neurogenesis in discrete areas of adult mammalian CNS. The subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus are the main neurogenic regions of adult brain that contain neural stem cells or neural progenitor cells, respectively. The basal rate of adult neural stem/progenitor cell generation within these discrete areas of the brain is modulated by several pathophysiological conditions, suggestive of a potential repair capacity of the damaged brain. CNS lesions attract transplanted stem cell populations from different sources, but little is known regarding the potential occurrence and involvement of endogenous neural stem/progenitor cells. The mobilization of possibly dormant endogenous stem/progenitor cells represents an attractive alternative strategy to transplantation. Here, we investigated if a population of stem/progenitor cells emerges in response to focal laser lesions in the visual cortex of young rats. In this lesion paradigm, enhanced synaptic plasticity in the penumbra region has been documented but it remained open whether neurogenesis contributes to this phenomenon.

The 473HD-epitope is expressed on the surface of neural stem/progenitor cells during development and maintained in the adult neurogenic brain regions. We recorded a fast ipsilateral upregulation of 473HD in callosal white matter areas and in the cortical layers I and VI. The immunoreactivity for 473HD colocalized with BrdU, vimentin, nestin and glial fibrillary acidic protein (GFAP) but not with microglial markers. Importantly, we could isolate cells from the lesion area that gave rise to selfrenewing, multipotent neurospheres. Their number was threefold higher compared with cells obtained from the intact contralateral hemisphere. Furthermore, the control spheres did not selfrenew and generated only astrocytes. Thus, we identified a rapidly appearing neurogenic progenitor population with radial glia characteristics *in vivo* and neural stem cell properties *in vitro* that potentially generate neurons *de novo* after defined laser lesions in the rat visual cortex. It is an interesting possibility that environmental cues released from the damaged tissue either instructs endogenous progenitors to proliferate and differentiate into specific cell types, or attract stem/progenitor cells from established niches to the lesion area. Although the cell fate and the underlying mechanisms are still unresolved, our observations suggest that the latent regenerative potential of the adult CNS may be far greater than previously assumed.

15.

Isolation and characterization of neural induction intermediates

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Keywords: embryonic stem cells, germ cell nuclear factor, neural induction, pluripotency

Embryonic stem cells lose pluripotency through a series of fate commitment events. We have isolated embryonic stem-derived cells that have gone through the earliest steps in neural commitment. GCNF is necessary for this commitment. Microarray profiling suggests both novel pluripotency-associated transcription factors and strategies to reprogram these cells back to pluripotency.

16.

Towards learning regulation, evolution and pluripotency from OMICs data

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Keywords: evolutionary inference, machine learning

Pluripotency, the ability to give rise to all cell types of the organism proper, is the foremost characterization of an embryonic stem cell. The network of regulation and interaction of protein and DNA that maintains the pluripotent state is only known to a small degree, and its elucidation is important for regenerative medicine, therapeutic applications, the reprogramming of somatic cells and even for aging research.

Multispecies comparison of the evolution of the regulatory regions of genes implied in pluripotency can yield predictions and insight into the network, based on genomic (transcription factor binding site estimation) and experimental (ChIP on CHIP and microarray) data. We showcase a tool that visualizes the evolution of putative regulatory elements using multiple alignment and tree reconstruction, and we will present an approach towards learning the regulators of pluripotency based on support vector machine learning from high-throughput data, beginning with microarrays.

Differentiation

20.

Isolation of chondrogenic progenitor cells from growth plate cartilage

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Keywords: cartilage, chondrogenic progenitor cell

Growth plate cartilage represents a differentiation system responsible for the axial long growth of bones. It can be subdivided into discrete zones representing sequential maturation stages of progenitor cells to terminally differentiated chondrocytes. The different zones are termed: resting, proliferating, pre-hypertrophic and hypertrophic zone. It is believed that stem cell-like cells within the resting/proliferating zone are committed to enter the differentiation and maturation process that is terminated by hypertrophy. These cells may therefore represent the optimal cell population to study chondrogenic differentiation processes and may determine a potential source of progenitor cells for cartilage repair processes.

Until now the isolation of viable cells from distinct growth plate cartilage zones was not feasible, mainly due to the lack of specific cell surface markers and appropriate isolation techniques. In order to discover these markers we developed a new micro-dissection method to isolate developmentally distinct chondrocytes from growth plate cartilage of 13 day old mice and assessed their transcriptomes by whole genome microarray technology. We identified several differentially expressed genes coding for cell surface proteins during chondrocyte maturation within the mouse growth plate. For ten of these candidate genes antibodies are commercially available which can be used in immunofluorescence studies and FACS analysis. Several of these antibodies bind specifically to the resting/proliferative, prehypertrophic/hypertrophic or hypertrophic zone of growth plate cartilage. By using an enzymatic based system to isolate viable cells from cartilage we could show that some of these antibodies also identify distinct populations within cell suspensions of mouse growth plate cartilage in FACS analysis.

At the conference we will present data that it is now possible to use a verified set of these antibodies labeled with various fluorochromes to discriminate and isolate cell populations from distinct maturation stages within growth plate cartilage. In the near future this approach will enable us to study the differentiation potential of cell populations from distinct maturation stages within the growth plate to identify the chondrogenic progenitor population. This cell population may play a central role in future tissue regeneration studies.

21.

Analysis of notch signaling in human embryonic stem cell-derived neural precursors

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Keywords: neural stem cells, Notch

Notch signaling is evolutionary conserved in vertebrate and invertebrate development, where it is known to regulate cell fate decisions. During vertebrate neural development in particular, Notch contributes to the maintenance of neural stem cells, while inhibiting neuronal differentiation. Our lab has succeeded in generating a neural stem/precursor cell population from human ES cells (hESNPs), which can proliferate stably and give rise to neuronal and glial progeny. These hESNPs represent a unique system to recapitulate early human neural development.

We investigated the role of Notch signaling in cultured hESNPs, and show quantitative expression data for the different Notch receptors and genes related to the pathway. Notch signaling is active in proliferating hESNPs and efficiently inhibited upon treatment with the γ-secretase inhibitor DAPT. Disruption of Notch signaling significantly decreases proliferation of hESNPs and induces neuronal differentiation, which is in agreement with previous mouse studies. We have cloned a cell-permeant version of the mouse Notch1 IC active domain (mNICD), which can be directly delivered to cultured cells by protein transduction. Our data indicate that transduction of DAPT-treated cells with mNICD is able to reinstate Notch signaling with a dose-dependent transcriptional regulation of downstream genes. This experimental system should provide a useful tool for studying the role of Notch signaling in human ES cells-derived neural stem cells in a time- and dosage-dependent manner.

22.

Isolation and differentiation of chromaffin progenitor cells from adrenal medulla

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Keywords: adrenal gland, chromaffin progenitor, dehydroepiandrosterone, differentiation

Chromaffin cells from adrenal medulla are the primary source of epinephrine and norepinephrine secretion. Both of chromaffin cells and the closely related sympathetic neurons have been assumed to derive from a common sympathoadrenal progenitor cell which develops from the neural crest during early embryogenesis. But unlike the sympathetic neurons, they are able to proliferate throughout the life span. The aim of this study is to isolate and to induce the differentiation from the chromaffin progenitor cells of adult bovine adrenal medulla. Chromaffin progenitor cells isolated in low-attachment condition grew into spheres and showed self-renewal ability. In molecular expression analysis, these chromospheres not only showed progenitor markers including Nestin and Sox1, they also expressed chromaffin cell markers such as TH and PNMT.

The adrenal androgens DHEA and dehydroepiandrosterone (DHEAS) are the most abundant androgens in human body. They are produced by the inner adrenocortical zone, zona reticularis, which in direct contacts with the adrenomedullary chromaffin cells. Our previous research showed that these steroids influence chromaffin cell proliferation caused by growth factors. We therefore addressed the question of a potential influence of these hormones on chromospheres differentiation. The expression of Nestin mRNA was reduced by treatment with DHEAS, and PNMT expression was induced by treatment with DHEA as well as dexamethasone. These data suggest that DHEA and DHEAS might play an important role in chromaffin cell development.

23.

Regional plasticity of long-term propagated human embryonic stem cell derived neural stem cells

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We have previously reported the generation of homogenous long term expandable human embryonic stem cell derived neural stem cells (lt-hESNSCs). Upon prolonged expansion in FGF2 and EGF these cells acquire a regional and phenotypic bias, which is characterized by posteriorization and the generation of predominantly GABAergic neurons. Here we address the question whether co-culture of these cells with different brain regions can influence and overcome these developmental restrictions. We chose shared medium and direct co-cultures, re-aggregation as well as transplantation onto organotypic slice cultures to explore whether cues from different brain regions can re-specify the regional phenotype of lt-hESNSCs. Cells were exposed to forebrain, mesencephalon, hindbrain and spinal cord of E12 mice, cortex and striatum, mesencephalon and cerebellum of P0 mice as well as hippocampal and cerebellar slices of 9-day-old rats. As readout we used human specific RT-PCR expression analysis of region specific transcription factors including FoxG1, Otx2, En1, Pax5, HoxA2 and Krox20. Remarkably, neither co-culturing with, nor transplantation onto different brain regions induced significant changes in region-specific gene expression. In contrast, exposure of lt-hESNSCs to Shh and FGF8 induced ventral midbrain markers and enabled the derivation of large numbers of TH-positive neurons. Moreover, retinoic acid treatment promoted further posteriorization with expression of more posterior Hox genes. Our data suggest that lt-hESNSCs retain a responsiveness to supraphysiological levels of single morphogens but are not easily amenable to regional respecification by tissue derived environmental cues.

Disclosures
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24.

Anxa5$^+$/Sca-1$^+$ cells derived from murine meningeal vasculature retain their perivascular mesenchymal stem cell-like phenotype *in vitro* and *in vivo*

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Keywords: mesenchymal stem cell, microvasculature, pericyte

Mutual interaction of endothelial cells and pericytes are crucial to form a stable microvascular network. Pericytes are critical for mediating the local blood flow, microvessel stability and may serve as a pool of vascular associated mesenchymal stem cells. Impaired pericyte coverage of the microvasculature is associated with the pathogenesis of atherosclerosis and diabetic retinopathy. However, little is known so far about pericyte biology mainly due to the lack of suitable purification techniques. Recently, we developed a method to isolated and characterize pericytes from mouse. Here we show that these populations display typical characteristics of pericytes by the presentation of pericyte markers NG2, Desmin, SMA, PDGFR-B as well as the stem cell marker Sca-1. Expression of endothelial (PECAM), hematopoietic (CD45) or myeloid (F4/80, CD11b) markers was not detectable. By contact with endothelial cells, pericytes stimulate angiogenic differentiation indicated by an increased expression of the endothelial marker PECAM and specific deposition of basement membrane proteins. Accordingly, *in vivo* grafts of pericytes on the chorioallantoic membrane of the quail embryo get highly vascularized and deposit basement membrane components. Our data demonstrate that functional pericytes can now be isolated and retain their unique perivascular mesenchymal stem cell-like character *in vitro*. These cells may therefore represent a general source of a vascular associated mesenchymal stem cell *in vivo*.

25.

Characterization and differentiation *in vitro* and *in vivo* of human cord blood-derived mesenchymal stem cells

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Keywords: human cord blood, mesenchymal stem cells, neuronal differentiation, transplantation

Human cord blood (HCB) is considered to be a new valuable tool in stem cell research. HCB contains in its mononuclear cell fraction a certain number of mesenchymal stem cells (MSC) that are able to proliferate and to differentiate *in vitro* into various cell types. MSC derived from HCB seem to have the same potential as the MSC derived from bone marrow. The objective of the project was to propagate HCB-derived MSC and to study their differentiation potential *in vitro* in cell culture systems and *in vivo* by transplanting them into the rat brain.

HCB probes were collected after informed consent of mothers according to the German guidelines for blood donation. Mononuclear cells were isolated from HCB by Ficoll density gradient centrifugation, and they were cultured in MSC medium. The MSC were growing as adherent cell fraction and they were cultured until their morphology changed from flattened to spindle-shaped. At this stage, the cells were pre-differentiated by exposure to bFGF- and EGF-containing medium for 1 week. Pre-differentiated cells were plated on fibronectin coated cover slips and cultured for further differentiation in defined media. Cells were fixed at several time points during differentiation (24 h up to 16 days) and processed for immunocytochemistry against different markers.

In parallel, a single cell suspension of either proliferating or pre-differentiated cells was stereotactically transplanted into the striatum and the hippocampus of Sprague–Dawley female rats to characterise their *in vivo* behavior. The rats were immunosuppressed during the entire experiment, and sacrificed at different time points. The brains were subjected to immunohistochemistry against different markers.

HCB samples exhibited a wide variety in survival patterns of mononuclear cells *in vitro*. In most cases, after three weeks in MSC culture medium, cell morphology reached a spindle-shape and cells started proliferating faster, whereas some probes kept growing in a flattened morphology. At this stage, bFGF and EGF exposure changed spindle-shaped cells morphology towards a neuronal phenotype. Cells were stained positive for Vimentin, Nestin, Neurofilament-200, Neurofilament-M, β-IIITubulin and GAD65. Transplantation results show short-(4 weeks) and long-term (12 weeks) graft survival in the striatum as well as in the hippocampus. Further results concerning the immigration patterns, integration and differentiation of the grafted cells will be presented at the conference.

Our results show that it is possible to isolate MSC out of HCB, to proliferate and to differentiate them into cells expressing stem cell markers as well as neuronal markers. The *in vivo* results suggest that HCB derived MSC have the potential to survive in the rat brain and can eventually be used as a cell source for replacement cell therapy in neurodegenerative disorders.

26.

Generation of functional neurons and glia from adult mouse germline-derived stem cells

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Keywords: *in vitro* differentiation, neural stem cells, pluripotency, spermatogonial stem cells

Recently, a population of multipotent stem cells has been derived from adult mouse testis (Guan, Nayernia *et al.*, *Nature* (440), 1199–203 2006). Similar to ES cells, these multipotent adult germline stem cells (maGSCs) show extensive self-renewal, express markers of pluripotency and can differentiate into derivatives of all three germ layers. A variety of medically relevant somatic cell types have been observed upon *in vitro* differentiation including heart, brain, muscle and skin cells. Here we describe the derivation of adherently proliferating multipotent neural stem cells (NSC) from maGSCs. These cells can be continuously expanded in the presence of epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2). Upon growth factor withdrawal they differentiate into neurons, astrocytes and oligodendrocytes. Characterization of maGSC-derived neurons revealed the generation of specific subtypes including GABAergic, glutamatergic, serotonergic, and dopaminergic neurons. Patch clamp recordings of passive and active membrane properties and postsynaptic currents in monolayer cultures demonstrated the functional maturation of maGSC-derived neurons. Within 2 weeks, they form functional networks that are spontaneously active and employ both glutamatergic and GABAergic synaptic transmission for synchronized oscillatory activity. MaGSC-derived NCSs also differentiate efficiently into oligodendrocytes, which undergo functional maturation, and ensheath host axons in cerebellar slice cultures derived from myelin-deficient rats. Thus, NSCs derived from maGSCs may provide a versatile and potentially autologous source of functional neurons and glia for biomedical applications.

Disclosures

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27.

Amniotic fluid-derived cells are pluripotent and exhibit neural differentiation characteristics

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Keywords: amniotic fluid, differentiation, glia, Oct-4, stem cells neurons, transplantation

In the search for cell populations suitable for cell replacement strategies, a variety of stem cell populations are being discussed. Recently, stem cells from the amnion or the amniotic fluid have shifted into the focus of interest, as it has been shown that, because of their plasticity, these cells have the potency to differentiate into a variety of cell types. However, at least three different cell types have been described to occur in the amniotic fluid. In order to select stem cells from the amniotic fluid and keep these cells in culture for some time, we have carried out a selection step by means of the magnetic-associated cell sorting using an antibody against the surface epitope CD117. With this procedure mainly epithelial-like cells and cells with mesenchymal characteristics could be obtained. analyzing stem cell characteristics, with RT-PCR transcripts for Oct-4, Nanog and Sox-2 could be detected. Furthermore, a small percentage of cells are additionally immunopositive for the stem cell markers SSEA-1 (CD15) and CD 90. Multipotency was assessed by characterizing their osteogenic and adipogenic differentiation potential. In

regard of a therapeutical application of these cells in diseases of the central nervous system, the neural differentiation capacity of amnion fluid derived cells was stimulated by cultivating cells in the presence astrocyte conditioned media as well as under hypoxia. Neural characteristics were analyzed using RT-PCR, ELISA as well as immunocytochemistry. After stereotactic transplantation of vector transduced cells from the amniotic fluid into the striatum of adult rats, neuronal and glial morphologies could be observed.

28.

Strategies for regenerative mesenchymal stem cell therapies of skeletal disorders: pathways to tendon formation – targeting of mesenchymal stem cells to cartilage tissues

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Keywords: cartilage, mesenchymal stem cells, targeting, tendon

Murine mesenchymal progenitors (C3H10T½) stably expressing bone morphogenic protein 2 (BMP2) and the constitutively active SMAD8ca, a member of the family of mediators transferring signals of TGF-β/BMP growth factors undergo differentiation into cells with tendon and ligament morphology *in vitro* and *in vivo*. In addition, adenovirally modified progenitors expressing both Smad8ca and BMP2 generate entire ectopic tendon–bone insertions with an osteotendinous junction exhibiting a fibrocartilage enthesis. Adenovirally modified progenitors expressing SMADca alone develop ectopic tendons without bony elements. These systems were used to repair a partial rat achilles tendon defect.

We also evaluated the capacity to target mesenchymal progenitors to degenerated cartilage surfaces. Chimeric receptors with the ScFv-collagen type II recognition domain as extracellular part and the transmembrane and cytoplasmic domains of BMP receptors type IA and II as signaling domains were hypothesized to initiate chondrogenic differentiation upon cartilage binding. Indeed, in murine C3H10T½ the presence of chimeric BMP-receptors is sufficient to induce a massive burst of chondrogenesis, which is dependent on the presence of both chimeric receptor types I and II expressed at a high level. Similarly, in a 3D culture system of human mesenchymal stem cells (MSCs), the combination of both chimeric receptors (II+IA) induces chondrogenic differentiation. Further studies will assess the therapeutic potential for these receptors to bring about cartilage regeneration upon cartilage-binding.

Disclosures

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29.

Modulation of embryonic stem cell-derived neuronal network activity via GABA receptors

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Key words: embryonic stem cell, neuron, neuronal differentiation, neuronal network, multielectrode array, gamma-aminobutyric acid

By applying the microelectrode array technology we recently described the development of functional neuronal networks generated from embryonic stem cells. In early stages of neuronal maturation (1–2 weeks), only single spikes and non-synchronous bursts of spikes were detected, whereas network activity as characterized by synchronously oscillating bursts of spikes was observed at later stages of maturation (>3 weeks). This electrophysiological maturation was paralleled by morphological changes, including increasing numbers of neuronal processes and presynaptic vesicles.

In the present investigation we analyse whether the modulation of excitatory or inhibitory synapses during early stages of neuronal maturation can elicit pre-mature synchronously oscillating bursts.

While the stimulation of glutamatergic transmission is not able to elicit synchronously oscillating bursts during the first 2 weeks of neuronal maturation, the inhibition of GABAergic activity can reliably produce synchronously oscillating bursts already after 2 weeks of neuronal maturation. These results show that the generation of synchronously oscillating bursts that define a functional neuronal network are not exclusively dependent on morphological criteria but also on the functional contribution of diverse neurotransmitter systems at different time points of neuronal maturation.

30.

From embryonic stem cell to neuronal network: development and pharmacological modulation of embryonic stem cell-derived neuronal network activity

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Key words: embryonic stem cells, neuronal differentiation, neuronal networks, multi-electrode array.

Embryonic stem (ES) cells can be differentiated into neurons of diverse neurotransmitter-specific phenotypes. While the time course of functional progression of ES cell-derived immature neural precursors towards mature neurons that fire spontaneous action potentials has been described in detail on single cell level by the patch clamp method, the temporal development as well as the pharmacological modulation of ES cell-derived neuronal network activity have not been explored yet. Neuronal network activity can be assessed by the microelectrode array (MEA) technology that allows to observe and record electrophysiological function of populations of neurons over several weeks or months in vitro. We could show here that ES cell-derived neural precursors cultured on MEAs developed synchronously oscillating neuronal networks over several weeks via distinct states of activity. This process was accompanied by an increase in the density of presynaptic vesicles and neuronal processes. Furthermore, we demonstrated that network activity was sensitive to synaptically acting drugs like gamma-aminobutyric acid or N-methyl-D-aspartic acid and their antagonists bicuculline or APV. We also show that magnesium-free salt solution supported synchronous burst activity and inhibited uncorrelated spike activity. These data indicate that populations of ES-derived neurons generate functional, pharmacologically influenceable neuronal networks, several weeks after the first onset of spontaneous spike activity of single neurons. Thus, the MEA technology represents a powerful tool to describe the temporal progression of stem cell-derived neural populations towards mature, functioning neuronal networks that can be applied to investigate pharmacologically active compounds.

31.

Differentation of hepatocytes from human embryonic stem cells

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Keywords: differentiation, gene expression, hepatocytes, human embryonic stem cells

The ability to increase the number of functional hepatocytes in a diseased liver would have obvious therapeutic potential. Recent studies suggest that cellular transplantation can be used to heal liver pathologies. Hepatocyte cells derived from mouse embryonic stem (ES) cells have been shown to integrate into liver tissue and produce albumin. In addition, when mouse ES cells were injected into mice with liver intoxication, hepatocytes that expressed liver-specific markers were developed. The main aim of this project is to examine how specific cytokines, growth factors and transcription factors support differentiation of human embryonic stem cells (hESCs) to hepatocytes in an efficient and reproducible manner. As an initial step in this differentiation protocol, Activin A and low serum concentration was used to promote definitive endoderm differentiation of the hESC line H9. This was then validated by analyzing the expression of the definitive endoderm markers SOX17, FOXA2, HNF4A at the mRNA and protein level. In the second step of the differentiation protocol we tested different conditions to induce hepatocyte differentiation. To achieve this we employed previously published protocol which led to differentiation into cells possessing morphologic and phenotypic features typical of hepatocytes. We also tested the effect of acidic fibroblast growth factor (aFGF) and SHH, which are important for hepatocyte differentiation during embryological development. Further experiments need to be performed to improve the efficiency of differentiation. Implementation of defined growth conditions should enhance differentiation to hepatocytes. Liver specific gene expression analysis and comparison between hepatocytes derived from hESC and fetal human hepatocytes have been used for further characterization of these cells.

32.

Investigating the *in vivo* and *in vitro* potential of different source-derived mesenchymal stem cells

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Keywords: mesenchymal stem cells, MRI, neuronal differentiation, transplantation

Parkinson's disease primary pathology involves degeneration and loss of dopaminergic neurons in the substantia nigra creating severe functional deficits in patients, while the currently available treatments are considered to be insufficient. Along these lines, cell replacement therapy has become a promising restorative treatment option.

The aim of the project is to develop cell culture protocols for mesenchymal stem cells (MSC) derived from umbilical cord blood (UCB) and umbilical cord's (UC) Wharton's Jelly and to differentiate them into neurons introducing a new potential source for cell replacement therapies.

MSC isolation from UCB was performed by Ficoll density-gradient centrifugation and cells were cultured in MSC medium in order to establish a MSC line.

MSC were successfully differentiated to adipocytes and osteocytes based on responsiveness to insulin/dexamethasone and β-glycerophosphat/ascorbic acid, respectively.

In order to promote neuronal differentiation, MSC were pre-differentiated for 1 week with EGF and FGF2 containing medium. Then cells were cultured from 1 up to 3 weeks in medium enriched with FGF8, BDNF, GDNF and retinoic acid. Cell differentiation response was compared between low and normal oxygen conditions. Fixed cells were processed for immunocytochemistry.

In parallel, MSC were obtained from the Wharton's Jelly of the UC. The veins and the artery were removed and the cord was filled with cell disassociating enzymes and incubated at 37°C for 30 min. The MSC were collected by washing the cord with medium.

To investigate the *in vivo* behavior of MSC, magnetic resonance imaging, MRI (*in vivo* imaging) was used. MRI labeling protocols with iron based contrast agents were optimized for MSC. The labeled MSC were stereotactically transplanted in the striatum of Sprague Dawley rats and the evaluation was analyzed in MRI scans.

To characterize the graft cell migration and differentiation, MRI scans were performed on different time points after the transplantation (15 days, 1 and 2 months).

In summary, MSC can be isolated and propagated from UCB and UC, whereas the outcome of proliferating UC-derived MSC was higher. Immunocytochemical analysis of differentiated cells showed neuronal marker expression.

Even though the iron labelling showed cytotoxic effects, it seems to be a suitable contrast enhancing method for MRI application. A detailed analysis of the MRI results will be presented at the meeting.

33.

The bioactive lipid sphingosylphosphorylcholine induces differentiation of (half of title missing in original)

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Key words: sphingosylphosphorylcholine, differentiation, lysophospholipids, mouse embryonic stem cells, promyelocytic leukaemia

Sphingosylphosphorylcholine (SPC) is the major component of high-density lipoproteins (HDL) in blood plasma. The bioactive lipid acts mainly via G protein coupled receptors (GPCRs). Similar to ligands of other GPCRs, SPC has multiple biological roles including the regulation of proliferation, migration, angiogenesis, wound healing and heart rate. Lysophospholipids and their receptors have also been implicated in cell differentiation. A potential role of SPC in stem cell or tumour cell differentiation has been elusive so far. Here we examined the effect of SPC on the differentiation of mouse embryonic stem (ES) cells and of human NB4 promyelocytic leukemia cells, a well established tumour differentiation model. Our data show that mouse embryonic stem cells and NB4 cells express the relevant GPCRs for SPC. We demonstrate both at the level of morphology and of gene expression that SPC induces neuronal and cardiac differentiation of mouse ES cells. Furthermore, SPC induces differentiation of NB4 cells by a mechanism which is critically dependent on the activity of the MEK–ERK cascade. Thus, the bioactive lipid SPC is a novel differentiation inducing agent both for mouse ES cells, but also of certain human tumour cells.

34.

Enhanced tissue integration of purified human embryonic stem cell-derived neuroblasts: overcoming inhibitory auto-attraction of stem cell-containing transplants

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Keywords: human embryonic stem, neural stem cells, neuronal migration, purified neuroblsts, tissue integration

Widespread migration and integration into the nervous system is a key prerequisite for the application of neural stem cells in neural repair. Typically, the efficacy of neural stem cell transplants in the adult brain and spinal cord is restricted by donor cell clustering and limited migration of neurons into the host tissue. The mechanisms underlying this restricted donor cell integration are barely understood. We used a genetic lineage selection approach to study the tissue integration of highly purified human embryonic stem (ES) cell-derived neuroblasts. Specifically, FACSorting was employed to select human neuroblasts from differentiating human ES cell-derived neural stem cells (NSCs) expressing EGFP under control of the human doublecortin promoter. When these EGFP-positive neuroblasts were transplanted into rodent CNS tissue, they exhibited extensive migration and functional integration. In contrast, transplants comprising NSC or a mixture of neuroblasts and NSC showed limited spread and integration into the host tissue. We reasoned that this phenomenon might be due to a chemoattractive effect of NSC on neuroblasts. Indeed, when studied in an *in vitro* transwell assay, human neuroblasts showed a pronounced chemoattractive migration towards undifferentiated NSC. Moreover, we found that purified human neuroblasts express receptors for chemotactic factors generated by NSC. First, *in vitro* data indicate that blockade of these chemoattractive mechanisms can abolish auto-attraction of neuroblasts by their more undifferentiated counterparts, resulting in enhanced emigration of immature neurons from mixed cell populations. These findings depict inhibition of autoattraction between NSC and neuroblasts as potential avenue to enhance migration and tissue integration of neuroblasts from NSC transplants.

Disclosure
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35.

L1 immuno-based isolation of human embryonic stem cell-derived neurons

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Keywords: cell adhesion molecule L1, hESC, immunoselection strategy, neuronal differentiation

Differentiation of human embryonic stem cells (hESCs) into defined lineages adds new perspectives to the development of cell-based therapies, human cell-based assay and screening application. We have recently established conditions for the derivation of stably proliferating neural stem cells (NSCs) from hESCs. Under appropriate culture conditions these cells differentiate into both neurons and glia. Here we describe an immunoselection strategy for the purification of immature neurons. As selection marker we used the neuronal cell adhesion molecule L1, which was found to be expressed in a subset of differentiating hESC-derived NSCs. After 7 days of growth factor withdrawal-induced differentiation, 5,2 ± 2% of the cells had acquired an L1-positive phenotype. Pre-differentiated cultures were labeledwith an antibody to L1 and subjected to preparative fluorescence-activated-cell-sorting (FACS), yielding a cell population comprising more than 96% L1-positive neurons. Upon replating and further differentiation, the FACSorted cells adopted neuronal phenotypes and extended MAP2ab-positive neurites. Moreover, physiological analysis of these cells revealed a population of electrically excitable neurons. During long-term differentiation on primary mouse astrocytes L1(+) selected neurons gained sodium and potassium currents and fired repetitive action potentials upon sustained depolarization by 9 weeks of cultivation. Furthermore, they displayed surface expression of AMPA/kainate and GABA-A receptors as prerequisite for the formation of glutamatergic and GABAergic synapses. Our results indicate that L1-based immunoselection effectively enables the generation of highly enriched cultures of human ES cell-derived functional neurons without genetic modification. Since the sorted cells can be frozen and thawed, they represent an attractive source of human neurons for biomedical applications such as compound screening and cell transplantation.

Disclosure
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36.

Cell-mediated RNAi gene therapy in the brain: gap junction-specific cell-to-cell transfer of short interfering RNA between astrocytes

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Keywords: astrocytes, gap junctions, RNAi

The therapy of CNS diseases is often hampered by the privileged status of the brain and its limited accessibility for drug treatment. As the CNS is characterized by an extensive network of astrocytes functionally coupled by different kinds of channels (gap junctions), astrocyte-mediated delivery of therapeutically active agents or modulation of the CNS gene expression profile represents an attractive alternative approach for sustained therapeutic effects. In this proof of concept study, we explore whether astrocytes engineered to overexpress siRNA can interfere with gene function in adjacent cells. Co-culture experiments demonstrate that Cx43 coupled astroglioma cells expressing a shRNA against EGFP(enhanced green flourescent protein) (shEGFP) can knock-down EGFP expression in their neighboring cells via gap junction-specific cell-to-cell transfer of siRNA. An obvious knock-down of EGFP expression in the recipient cells was observed after 6 days of co-cultivation. This transcellular effect could be prohibited using the gap junction inhibitor 18α-glycyrrhetinic acid (AGA). Moreover, we established a model system consisting of primary EGFP expressing neural stem cell-derived astrocytes as recipient cells and shEGFP-expressing ES cell-derived astrocytes as donor cells. Astrocytes from both populations express high levels of Cx43 and show extensive gap-junction mediated coupling *in vitro*. The delivery of functional siRNA from donor astrocytes to the astrocyte syncytium may therefore lead to a novel ES cell-based principle of cell-mediated loss-of-function gene therapy in the brain.

37.

Human umbilical cord blood cells: proliferation, differentiation and cytokine secretion *in vitro*

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Keywords: conditioned medium, cytokines, differentiation, proliferation, umbilical cord blood

Human umbilical cord blood (hUCB)-derived mononuclear cells were previously shown to exert therapeutic effects in a number of animal models of nervous system impairments.

However, the molecular and cellular mechanisms underlying the functional improvement are still unclear. As neuronal differentiation of transplanted cells seems to be a rare or absent event *in vivo*, we propose secondary mechanisms, which might be responsible for the therapeutic effects. We investigated the potential of hUCB-derived mononuclear cells *in vitro* to proliferate, differentiate, and secrete factors possibly beneficial for the host brain tissue *in vivo*.

Using a succession of distinct culture media, mononuclear cells were stimulated by growth factor combinations. Cells responded with proliferation, as assessed by detection of the Ki-67 protein, and were capable of differentiating into neuron- and glia-like cells with a simultaneous reduction of expression of the hematopoetic marker protein CD45. Most importantly, in response to either growth factor combination, cells were shown to secrete various cytokines. Significant amounts of cytokines were detected for proteins of the interleukin family, growth factors and chemokines.

Although capable of incipient differentiation, cytokine secretion of hUCB-derived mononuclear cells envisages the potential of an indirect effect *in vivo*. Most factors detected in conditioned medium are renowned for their anti-inflammatory, neuroprotective, angiogenic or chemotactic action. Thus, cytokines secreted by human mononuclear cells might be suitable candidates mediating functional recovery after hypoxic-ischemic brain injury.

38.

Human mesenchymal stem cells for therapy of inherited liver disease

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Keywords: hepatocyte, mesenchymal stem cells

Mesenchymal stem cells (MSC) derived from human bone marrow have been previously shown to differentiate into hepatocyte-like cells, and may have great potential for cell-based therapy of liver disease. To study the effect of MSC after transplantation into animal models of liver disease we have isolated MSC from human bone marrow, and characterized these cells by flow-cytometry and real-time RT-PCR analysis. MSC expressed typical surface markers ($CD45^-$ $CD34^-$, $CD14^-$, $CD29^+$, $CD90^+$, $CD44^+$, $CD166^+$ and $CD105^+$), and showed low expression of marker genes associated with pluripotency (*Oct4* and *Nanog*). However, upon hepatogenic stimulation *in vitro*, MSC differentiated into hepatocyte-like cells. Cell morphology changed from a spindle-like to a polygonal-shape typically observed in adult hepatocytes. By RT-PCR analysis, a significant change in the expression of several markers occurred. Importantly, expression of early (AFP, CK19 and CK7) and late hepatocyte markers (CYP, CX32, CK18 and albumin) were upregulated by several magnitudes of relative gene expression levels. Our results suggest that the *in vitro* generated hepatocyte-like cells carry most functions of adult human hepatocytes. In order to modify gene expression of these cells we constructed retroviral vector for transgene expression, for example, of the copper transporter gene ATP7B. This gene is mutated in patients having Wilson disease (WD), which leads to copper overload of liver and other organs. To define the function of hepatocyte-like cells *in vivo* we are currently preparing such cells for transplantation into a rat model of WD. Therapeutic effects by transplanted cells will be determined by clinical and biochemical parameters. In conclusion we generated a cell line that carries many functions of adult human hepatocytes. This cell line may have clinical applications.

39.

Progenitor cells from the neural crest: microarray analysis displays differences in cell cultures derived from pulp and pad-like tissue of immature human third molar teeth

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Keywords: microarray, neural crest, progenitor cells, third molar teeth

Human third molars are the last developing teeth and are often extracted. As such they can serve as an easily accessible model for human tooth development. Cells responsible for tooth development originate from the cranial neural crest. We have characterized different compartments of impacted third molars by specific signatures of gene expression. From two of the compartments, the pulp proper and the pad-like tissue (PLT), we derived cell cultures. In order to see how the influence of the tissue diversification is translated and carried on in cell culture we analyzed their gene expression profile by microarray analysis. Signatures were found to be significantly different between both groups and varied between 2218 and 6928 in total numbers on the basis of P = 0.01. Since cRNAs gained from cells derived from pulp and PLT were labeledprior to hybridization with Cy5 and Cy3, respectively, we were able to assign all red and green signals to pulp or PLT representative signatures. In all, representative signals for cells derived from pulp ranged from 749 to 3405 (red) and from 1002 to 3523 for PLT signatures (green) depending on the samples analyzed. Focusing on intersections within all derivatives from pulp or PLT, only few genes were significantly matched in color. For all cell cultures derived from pulp, 11 signatures overlapped. Among these, annotated genes were WNT16 (wingless-type mouse mammary tumor virus [MMTV] integration site family, member 16), interferon (α, β and ω) receptor 2 (IFNAR2), microphthalmia-associated transcription factor (MITF) and methyl transferase like 7A (METTL7A). Representative for all cell cultures derived from PLT were eight signatures with annotated genes, such as semaphoring VIA (SEMA6A), myogenic factor 5 (MYF5), tyrosine aminotransferase (TAT) and IQCA-protein (IQCA). A 2D clustering of the

array-data resulted in the formation of two main clusters according to signatures that showed, at least in three of four cases, a significant 'pulp or PLT character', in other words, were significantly red or green ('cluster view'). The same genes as listed above for intersection formation could be found as PLT or pulp representatives via cluster analysis.

Therefore we propose that already in the early stage of tooth development neural crest-derived progenitor cells obtained from PLT and developing pulp show substantial differences in their respective gene expression profiles on a whole genome scale. This indicates a compartment-dependent commitment of the isolated cells.

40.

Development of unrestricted somatic stem cells from human umbilical cord blood into the neuronal phenotype *in vitro*

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Keywords: dopamine, nurr1, tyrosine hydroxylase, unrestricted somatic stem cells, voltage-gated sodium channels

Human umbilical cord blood stem cells are increasingly considered in cell-based therapeutic strategies for regeneration of different tissues. One subpopulation, unrestricted somatic stem cells (USSC), are a form of pluripotent, neonatal, non-hematopoietic stem cells which have the potential to differentiate into the neural lineage. However, the molecular and functional characterization of the neural phenotype and evaluation of the degree of maturity of the resulting cells are still lacking. In this study we examined the neuronal differentiation and maturation of USSC induced by XXL-medium, a defined composition of growth and differentiation factors. Specially, we investigated the expression of different neuronal markers and their enrichment in USSC cultures during XXL-medium incubation. Further, differentiation into the dopaminergic phenotype was examined. In addition, the induction of Nurr1, a factor regulating dopaminergic neurogenesis, and an enrichment of USSC expressing tyrosine hydroxylase, the key enzyme of dopaminergic neurons, could be demonstrated.

Functional differentiation of USSC was confirmed by patch-clamp recordings that revealed functional voltage-gated sodium-channels in laminin pre-differentiated USSC. Additionally, high performance liquid chromatography was performed showing synthesis and release of the neurotransmitter dopamine by USSC-derived cells, thus correlating well with the detection of dopaminergic markers, transcripts and proteins.

In conclusion this study provides novel insight into the potential of unrestricted somatic stem cells from human umbilical cord blood to acquire a neuronal phenotype and function.

Role of the biological niche

47.

Absence of functional GABAA receptors in transit-amplifying stem cells of the early postnatal subventricular zone

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Keywords: γ-aminobutyric acid receptors, EGFR, PSANCAM, subventricular zone, transit-amplyfing

In the subventricular zone neurogenesis is regulated by the coordinated proliferation and differentiation of different precursor types: slowly proliferating type B cells, rapidly dividing transit-amplifying type C cells and type A neuroblasts. Previous evidence suggests that γ-aminobutyric acid (GABA) released by neuroblasts provides a feedback signal inhibiting the proliferation of type A and B cells by activation of GABAA receptors, while the effects of GABA on type C cells are unknown. Here we used levels of EGFR expression and lineage-specific markers to purify stem cells and neuroblasts from the postnatal subventricular zone. Clonal assays and analysis of mice lacking expression of orphan receptor Tlx antigen showed that cells expressing high levels of EGFR (EGFRhigh) directly correlated with stem cell activity *in vitro* and *in vivo*. Analysis of antigen expression and BrdU incorporation *in vivo* revealed that more than 80% of the isolated cells represented putative type C cells. Although isolated EGFRhigh cells and neuroblasts were both PSANCAM positive, they displayed profound functional differences. Neuroblasts but not stem cells expressed GABA and Doublecortin and showed a fast intracellular Ca^{2+} increase upon acute membrane depolarization or application of GABA. Electrophysiological measurements also revealed different passive membrane properties and resting potentials in the two cell populations. Neuroblasts and stem cells both showed TEA-sensitive outwardly rectifying K^+ currents, but at different current densities. Strikingly, only neuroblasts expressed voltage-activated Ca^{2+} channels and displayed GABAA receptors mediated Cl^- currents. Furthermore, selective activation of GABAA and GABAB receptors did not affect stem cells' clone-forming capability nor their *in vitro* proliferation rate. Thus, transit-amplifying stem cells derived from the early postnatal subventricular zone do not express functional GABAA receptors and GABA does not directly modulate their proliferation.

48.

Adhesive and migratory properties of laminin-511/521 in the human endosteal stem cell niche

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Keywords: cell adhesion, extracellular matrix, proteolytic microenvironment, stem cell mobilization, stem cell niche

In the bone marrow stem cell niche, osteoblasts lining the endosteum are of major importance in supporting hematopoietic stem cell maintenance and controlling self-renewal. Here, we report on the expression of different laminin isoforms by primary osteoblasts and their functional interactions with isolated human hematopoietic stem and progenitor cells (HSPCs). Using laminin chain-specific antibodies in immunofluorescence staining and co-immunoprecipitation analyzes, laminin isoforms containing an α3 chain (LM-311/321) as well as an α5 chain (LM-511/521) were found to be expressed by human osteoblasts. Interestingly, laminin-511/521 was also synthesized by human HSPCs themselves as observed by RT-PCR, western blotting and immunofluorescence staining. LM-511/521 is a strong adhesive substrate for human $CD34^+$ HSPCs, and this interaction

was found to be mediated by β1-integrins. Furthermore, HSPCs adhered strongly to the peptide ASKAIQVFLLGG corresponding to a sequence of the human laminin α5 LG2 domain. This interaction could also be inhibited by function-blocking anti-β1-integrin antibodies suggesting that the indicated sequence represents an actual cell binding site for HSPCs.

We had recently shown that the matrix-metalloproteinase MMP-8 (collagenase-2) is released into the microenvironment during normal, induced or pathological stem cell mobilization. Treatment of LM-511/521 with activated MMP-8 resulted in a strong decrease of $CD34^+$ progenitor cell attachment to this digested laminin isoform. HSPCs also strongly adhered to primary osteoblasts in a β1-integrin-dependent manner. Digestion with activated MMP-8 significantly diminished cell adhesion of HSPCs to the adherent layer of primary osteoblasts. Further on, we analyzed whether LM-511/521 had an influence on cell migration of HSC. Interestingly, a strong chemotactic activity could be observed for LM-511/521 after digestion with the collagenase MMP-8.

Taken together, these data suggest that LM-511/521 can represent an important component of the extracellular matrix of the endosteal stem cell niche. Under steady-state conditions, HSPCs adhere to the intact LM-511/521. During mobilization, however, when MMP-8 is released into the proteolytic microenvironment of the bone marrow, MMP-8-digested LM-511/521 can help to mobilize stem cells out of their niche.

49.

Nongenetic, reversible Notch activation by cell-permeable recombinant Notch protein

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Keywords: neural differentiation, Notch, protein transduction

Embryonic stem cells have become a major focus of scientific interest as a potential source of transplantable cells for regenerative medicine. Notch signaling has been recently reported to play an important role during neural specification of undifferentiated, pluripotent embryonic stem cells. Thus, modulating Notch signaling *in vitro* may represent an important approach to guide differentiation into neural cell types. However, thus far the manipulation of Notch signaling in embryonic stem cells is limited due to poor over-expression of Notch ligands and/or activated Notch intracellular domain (NICD). To circumvent unwanted side effects of co-culturing with ligand-expressing cells or introduction of foreign DNA we made use of the protein transduction technology. Recently, we have devised a Cre protein transduction system, which permits the direct delivery of biologically active Cre protein to human and murine embryonic stem cells and their neural progeny (Haupt *et al.* 2006, Nolden *et al.* 2006). We have now established a transducible Notch protein by fusion of the NICD sequence to the TAT transduction domain. For that we expressed a TAT-fusion of mouse Notch1–ICD in bacteria and purified the recombinant protein from bacterial lysates via a fused His-tag. The purified recombinant protein exhibited over 70% purity and was shown to be stable under cell culture conditions. Identification of the recombinant TAT-NICD protein was carried out employing immunoblot analysis and immunocytochemistry.

Our data indicates that TAT-NICD accelerates cell cycle progression in somatic cells and restores downstream gene expression after inhibition of endogenous Notch signaling in neural precursors. Moreover, we present data on the effect of NICD transduction on terminal neural differentiation of these cells.

Disclosures
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50.

FGF-dependent signaling in neural stem cells requires carbohydrates of the chondroitin sulfate glycosaminoglycan class and a normal sulfation pattern

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Keywords: extracellular matrix, maintenance, neural stem cell niche, neurosphere

During CNS development neural stem cells (NSCs) are found in the ventricular and subventicular zone of the neuroepithelium that are referred to as germinal layers of the CNS or neural stem cell niche. In the adult brain the ventricular zone has disappeared and the subventricular zone has lost its aibility to sustain neurogenesis with the exception of the subependymal layer of the lateral ventricles where neurons and glia continue to be generated life-long. Chondroitin sulfate glycosaminoglycans (CS-GAGs) represent defined linear sugar residues that are covalently attached to various proteoglycans of the lectican family. They are constituents of the extracellular matrix and expressed in the developing and adult neural stem cell niche. The complexity of CS-GAGs is largely increased by the activity of chondoitin sulfate transferases that transfer sulfate groups to defined positions. In this way chondoitin sulfate transferases generate a complex environment that is hypothesized as the chondroitin sulfate code, and which harbors distinct growth factor binding sites.

Here we report that most chondoitin sulfate transferases are expressed in the developing and adult NSC niche as well as in NSCs grown as neurospheres. We investigated the functional role of these sugar residues by either removing the CS-GAGs entirely or by supressing their sulfation with sodium chlorate. Both treatments caused a marked reduction in the aibility of NSCs to form neurospheres in the presence of FGF. This was recorded after treatment *in vitro* and after injection of chondroitinase ABC *in vivo*. Purification of CS-GAGs from neurosphere conditioned medium revealed that this class of carbohydrates binds FGF in biochemical assays and is essential for FGF-dependent self-renewal of NSCs but not for their differentiation or cell fate choices. Interestingly, FGF binding sites overlap with the 473HD-epitope, which is a defined CS-structure that allows for NSC isolation. In addition, the antibody 473HD that recognizes this epitope also interferes with neurosphere formation *in vitro*. Therefore, we conclude that distinct CS structures including the 473HD epitope are necessary for FGF-dependent growth of NSCs and their maintenance.

51.

Tenascin C induction gene trap screen in neural stem cells identifies RBS as a target of extracelluar matrix signalling

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The stem cell niche constitutes the fundamental functional entity that integrates physiological signals and in this way controls the balance between stem cell maintenance and differentiation. Tenascin C (Tnc) represents a major extracellular matrix (ECM) glycoprotein in the germinal layers of the developing brain and of the adult neural stem cell (NSC) niches. Tnc is an alternatively spliced, modular glycoprotein of ECM that occurs in at least 20 different isoforms. It influences the behavior of NSCs during embryonic development by modulating their growth factor responses as well as the correct temporal expression of the epidermal growth factor receptor. However, it remains incompletely understood which genes are regulated by Tnc signaling in NSCs and how putative target genes modulate the responses of NSCs to intrinsic and/or extrinsic signals. To identify and characterize genes that are regulated by Tnc, we applied the induction gene trap technology to mouse NSCs grown as free-floating neurospheres. We generated and screened a library of 500 clones, in which approximately 1% of the integrations responded to Tnc treatment. We identified three integrations by 5`-RACE-PCR as a dynein light chain, a guanine nucleotide–exchange factor and a RNA binding protein (RBS), with the latter two target genes being expressed in the germinal layers of the developing forebrain. The Tnc-dependent down-regulation of the target genes was independently validated by RT-PCR and western blot analysis in nontransfected NSCs. In the detailed comparative in situ hybridisation analysis of RBS- and Tnc-expression in the

NSC niche, we recorded that strong Tnc signals correlated with decreased RBS mRNA expression, suggesting that Tnc might also down-regulate RBS in vivo. In a functional approach, we found that overexpression of RBS in NSCs modulates the alternative splicing pattern of Tnc by increasing the larger isoforms in a phosphorylation-dependent manner. These findings are suggestive of a regulatory feedback mechanism involving RBS, where Tnc plays an instructive rather than a permissive role within the NSC niche by modulating its own isoform complexity and composition. Taken together, our induction gene trap approach allows for novel insights into NSC biology will open new avenues for further basic and translational research.

52.

Asymmetry in human third molars during development: implications for different niche conditions

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Keywords: development, gene expression, neural crest, tooth

Tooth development is driven by a complex crosstalk between epithelium and neural crest-derived mesenchyme via signaling molecules, transcription factors and influences of the extracellular matrix. Despite knowledge of tooth development in rodents, the knowledge on gene expression important for a comprehensive tooth development in humans remains fragmentary. The aim of this study was to gain more insight into the molecular mechanisms that control differentiation of the human tooth, and to clarify whether different compartments (niches) show inherent differences in their expression levels of stemness markers due to different cell populations.

Two developmental stages of human impacted third molars were divided into the operculum, periodontal ligament, developing pulp and – as a new approach – the pad-like tissue beneath the pulp. We characterized the expression level of all compartments by real-time PCR of 16 different genes.

The expression of MSX2 and HNK1 in all compartments suggests their ectomesenchymal origin. With regard to markers for ectomesenchyme and tooth development every single compartment held its own signature of gene expression obviously reflecting the fact that during development differentiation of compartments were about to diverge. The expression patterns found shift in the course of development underscoring the relevance of these genes involved in human tooth development. The expression of markers, characteristic of stem cells, such as NANOG or OCT4 pointed to high potent features. Additional genes associated with characteristic features of stem cells, such as c-KIT and ABCG2, could be detected. Asymmetric expression of genes known to be components of the extracellular matrix like MEPE or PERIOSTIN indicate differences in niche conditions for resident stem/progenitor cells. Interestingly, the expressions of OCT4 and NANOG were concentrated within different tooth compartments. This would suggest that progenitor/stem cells residing in tooth tissue split up into different sub-populations. In summary, we conclude that the gene expressions found, at least partially, correspond to the determination of niche conditions for progenitor cells residing in the respective compartments.

Tissue regeneration

53.

Retrospective birth dating of cardiomyocytes in the adult human heart

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Keywords: birth dating, cardiomyocytes, human, regeneration

The ability of myocardial cells to regenerate in the adult mammalian heart has been a subject of controversy over the past decades. Originally, the heart was viewed as an organ that lost its ability to regenerate. However, this prevailing opinion has needed reconsideration recently in light of studies indicating that some degree of heart muscle cell turnover was indeed present under normal as well as under pathologic disease conditions. Traditional methods used for dating cells are limited or not appropriate for human use. We have taken advantage of the integration of 14C (radiocarbon), generated by nuclear bomb tests during the Cold War, into DNA in order to establish the age of cardiomyocytes in the human heart. Using flow cytometry, the nuclei of both cardiomyocyte and non-cardiomyocyte populations were isolated and the purified DNA was subjected to 14C analysis. We will present preliminary data on turnover in human cardiomyocytes. Turnover kinetics and DNA synthesis of human cardiomyocytes will be discussed.

54.

Human dental neural crest-derived progenitor cells can serve as a model for bone formation under *in vitro* conditions

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Keywords: bone, neural crest, stem cells, tissue engineering, tooth

Current evidence suggests that the connective tissue of the human tooth contains a distinct population of neural crest-derived progenitor cells (dNC-PCs) which have been proven to give rise to a wide range of specialized daughter cells such as neurogenic, chondrogenic and osteogenic lineages when outside their dental signal network [1]. The ability of dNC-PCs to develop engineered bone in a self-propagating culture system was further investigated. dNC-PCs were initially seeded in growth medium (DMEM-LG) containing 10% fetal calf serum. When reaching subconfluency, cultures were further grown in osteogenic differentiation medium, in other words, growth medium supplemented with dexamethasone, ascorbic acid-2 phosphate and β-glycerol phosphate. Differentiation of dNC-PCs as well as matrix formation and its mineralization process were studied up to 150 days of culture. After 14 days of osteogenic stimulation, cells had formed a multilayer with the development of several distinct nodules (spheric centers) scattered within the culture. Flat cells covering the surface (i.e. outer cells) as well as cuboidal cells trapped within the layer (i.e., inner cells) could be observed. With prolonged culture time, the culture system was dynamically developed by both cell populations. An organic matrix that is not mineralized initially was secreted by the inner cells. These cells showed an osteoblast-like phenotype. They are probably derived from the outer cells that represent osteoblast-like precursor cells. The outer cells were not responsible for a net increase in matrix. In the course of stimulation (after 21–28 days), the multilayer detached from the plastic substrate and contraction of the layer was observed resulting in a

3D mass. Once contracted, the inner osteoblast-like cells underwent apoptosis (proven by the terminal deoxynucleotidyl transferase dUTP nick end labeling technique). As a result of apoptosis, calcification of the matrix was observed converting the soft mass into a hard mass. Outer cells were essential to the maintenance of the compacted mass while persisting as a sheath of living cells on its surface; however, proliferation slowed down. Since dNC-PCs respond to osteogenic stimulation as shown in our study, they can serve as an *in vitro* model for bone formation. In this model, not only can the conversion of precursor cells into osteoblasts be studied, but also processes in bone matrix organization related to osteogenic cells. These results suggest that dNC-PCs could also be used for tissue engineering for the bone defects of the maxillofacial region.

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55.

β-cells derived from extrahepatic biliary epithelium

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Keywords: β-cells, diabetes, insulin

Transplantation therapies for diabetes have been explored for many years, but islet cell transplants are limited due to donor supply. One way forward is to produce β-cells *in vitro* for transplantation. This approach may eventually become possible with islet progenitor cells, adult stem cells, embryonic stem cells or existing β-cells.

An alternative possibility is to generate pancreatic tissue, containing β-cells, from some other tissue already present in the body.

We have focused on the possibility of generating β-cells from cells present in the extrahepatic biliary epithelium. The reason for focusing on biliary epithelium is twofold. First, deleting Hes1, a downstream target and mediator of Notch, causes the conversion of biliary epithelium to pancreatic tissue [1]. Second, we recently found a small population of extra-pancreatic endocrine cells within, or adjacent to, bile ducts in the hilar region of adult mouse liver [2]. These observations together suggest that extrahepatic bile ducts and pancreatic endocrine cells have a similar transcription factor profile. We have characterized the β-cells present in extrahepatic bile ducts and analyzed their cellular origin. Our results suggest the extrahepatic foci of endocrine cells contain bone fide β-cells generated from the developing liver region. The appearance of endocrine cells in the extrahepatic ductal epithelium suggests it may be possible to reprogram biliary epithelium to beta cells for the treatment of diabetes. We have now extended our initial observations to develop new culture systems for the maintenance of embryonic biliary epithelium and adult gall bladder epithelium. We are currently investigating the potential of exogenous factors and ectopic expression of pancreatic transcription factors to induce or promote expansion of the β-cell population in extrahepatic bile ducts and gall bladder epithelium.

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56.

Cytokines highly influence the migratory activity of murine hematopoietic stem and progenitor cells

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Keywords: cell migration, *ex vivo* expansion, hematopoietic stem cells, SDF-1a

The self renewing capacity of hematopoietic stem and progenitor cells (HSPCs) has stimulated investigations of *ex vivo* stem cell expansion to improve the quality of stem cell transplants. Efficient engraftment of HSPC requires homing to bone marrow, depending on directed migration along a gradient of stromal cell-derived factor (SDF)-1α. Thrombopoietin (TPO), Flt3-Ligand (FL), stem cell factor (SCF) and interleukin 11 (IL-11) are able to stimulate amplification of primitive murine HSPC *in vitro*. Murine Lin-c-kit+ HSPC were cultivated in the presence of twelve combinations of these hematopoietic growth factors to elucidate their effects on migratory capacity. Analysis of locomotory behavior was carried out in a 3D collagen matrix using time lapse video microscopy. Each cytokine combination has a distinct effect on the SDF-stimulated and spontaneous migratory activity of murine HSPC, which allowed for their grouping according to the intensity of the SDF-response. The highest migratory response to SDF was observed for FL and SCF (FS) cultivated cells, while SCF, TPO and Il-11 (STI) cultivated HSPC did not respond to SDF-stimulation. However, fold expansion as well as CXCR4 expression levels do not correlate with migration. Astonishingly, for example, STI cultivated non responding cells we observed an inhibitory effect of SDF on migratory activity. Depending on the cytokine combination, we observed differential involvement of the MAPKp42/44 cascade and the PI3K cascade downstream of CXCR4. The SDF-1α induced migration of FSTI and F cultivated HSPC strongly depended on activation of PI3K and MAPKp42/44, whereas the PI3K as well as the MAPKp42/44signal transduction pathways were not involved in SDF-1α induced migration of FST, FTI or FI cultivated HSPC. Our results show that hematopoietic cytokines have a dramatic impact on the intracellular signaling machinery of expanded murine HSPC and their SDF-stimulated locomotory activity, suggesting careful consideration of cytokine supplementations for *in vivo* transplantation setups.

57.

In vitro investigation of axon growth promoting properties of adult human mesenchymal stromal cells and their interactions with a microstructured and highly orientated 3D collagen scaffold

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Keywords: biomaterials, mesenchymal stromal cells, nerve regeneration

Traumatic injury to the CNS or PNS leads to functional loss by disrupting long-distance projecting axons in white matter tracts or in peripheral nerves. The lack of regeneration following spinal cord injury is mainly caused by the presence of cystic cavitation and a hostile environment at the lesion site. Limits to the success of peripheral nervous system (PNS) axon regeneration are influenced by the size of the lesion gap and the material used to bridge it. Although a range of experimental intervention strategies/materials for bridging spinal cord injury have been investigated, an ideal substrate has yet to be identified. For PNS injuries, autografts remain the 'gold standard'; for large lesions and a bioengineered scaffold capable of replacing such autografts is still to be developed.

A cell population suitable for an axon growth-promoting, tissue-engineering strategy is adult human bone marrow-derived mesenchymal stromal cells (hMSCs). We have investigated the axon growth promoting properties of isolated hMSCs in simple 2D cultures using freshly dissociated adult rat dorsal root ganglion (DRG) neurons. Furthermore, the suitability of these cells in contributing to a 3D tissue engineered scaffold has been investigated *in vitro* by seeding hMSCs onto a highly orientated and micro-structured type I collagen scaffold. Samples were fixed in 4% paraformaldehyde after 1 day and 4 weeks, cryosectioned and processed for immunohistochemistry.

Confluent cultures of hMSCs tended to grow in an orientated manner. Interestingly, neurite outgrowth of dissociated DRG neurons followed the longitudinal orientation of the hMSCs, suggesting that these cells were not only capable of supporting neurite growth but were also capable of directing it. Non-oriented outgrowth of neurites was observed on PLL/laminin coated tissue culture surfaces, serving as a control.

As potential cell carrier in a 3D tissue-engineering strategy, we have been investigating a highly porous type I collagen scaffold that contains densely packed, large diameter (100 µm) channels. Seeding of the hMSCs on the orientated collagen matrix revealed an even distribution that persisted over time. No signs of apoptotic, fragmented cell nuclei could be observed with the DAPI counterstain. The *in vitro* demonstration of ability of hMSCs to support and guide axonal regeneration, as well as the relatively uniform seeding of such cells into a highly orientated type I collagen scaffold suggest that this combination of stem cells and biodegradable scaffold merits further investigation into its potential for supporting tissue repair and axon regeneration.

Disclosure

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58.

Reliable derivation of *in vivo* preimplantation embryos from the marmoset monkey (*Callithrix jacchus*)

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Keywords: cell replacement therapy, embryo, embryonic stem cell, non-human primate, preclinical

The common marmoset monkey (*Callithrix jacchus*) is widely used as an experimental animal model in biomedical research. This small non-human primate provides a valuable link between rodent species and the human and serves as a model for diseases such as Parkinson's disease, diabetes and multiple sclerosis. Cell-replacement therapy using differentiated cells derived from embryonic stem cells (ESCs) holds tremendous potential to cure cell degenerative diseases. Thus, the creation of marmoset ESC lines is crucial to evaluate the potential of ESCs as a cure in a preclinical setup.

To generate new ESCs it is implicitly important to obtain pre-implantation embryos up to the blastocyst stage. The first step in the generation of novel ESC lines at the German Primate Centre (Deutsches Primatenzentrum [DZP]) is regularly performing a minimally invasive uterus flush in marmoset monkeys. The ovulatory cycle of *Callithrix jacchus* is similar to the human in length. However, the follicular phase is slightly shorter (8 days) and the luteal phase longer (20 days). Embryo implantation is supposed to take place 11 days after ovulation. To determine the optimal time for obtaining preimplantation embryos the monkeys' ovarian cycles were recorded. Monitoring was performed by measuring levels of plasma progesterone using an in-house ELISA. The female marmosets were coupled with castrated or vasectomized males and, in the meantime, their ovarian cycles were determined. After 2 months of monitoring, the females were re-coupled with fertile males and uteri were flushed during up to four ovulatory cycles. Before the flush the animal was sedated with a ketamin/xylazin mix (2.5 mg/kg: 2.0 mg/kg) and received a small incision (~1 cm) in the mid-line of the ventral abdominal wall. The ovaries and uterus were taken out of the abdominal cavity and the uterus was pinched off by Biemer vascular clips at the tubes and the cervix. The uterus was penetrated by two cannules from opposite sides. A total of 2 ml of cell culture medium was slowly injected through one cannule into the uterus. The other cannule served to collect medium containing the embryos, which were immediately transferred to embryo culture.

Utilizing 13 animals so far, 35 flushes have been performed obtaining 40 embryos of different developmental stages (22 blastocysts, 16 morulae and two zygotes). These data prove that we have established a reliable method to obtain natural preimplantation embryos from a non-human primate. These embryos are currently used to establish novel ESC lines which will serve to conduct basic developmental studies, as well as to establish cell-replacement therapy approaches in a clinically relevant non-human primate model.

59.

Corneal epithelial stem cell deficiency and its therapy with transplantation of *in vitro* cultivated limbal stem cells

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Keywords: adult stem cells, corneal epithelium, limbal stem cells, stem cell therapy

Intact corneal epithelium is essential for normal vision. Epithelial defects can heal only in the presence of stem cells (SC) that are located in the corneal marginal (limbal) zone. These stem cells may be destroyed by severe damage to the ocular surface from several conditions. This results in limbal stem cell deficiency (LSD) which is characterized by in growing conjunctival epithelium (pannus), chronic inflammation and impaired vision. Transplantation of *in vitro* cultivated limbal SCs has been recently developed to treat LSD.

The objective of this study was to characterize the changes taking place in the cornea during LSD and the effect of SC transplantation.

A total of 14 patients with LSD were treated by transplantation of limbal SCs expanded on amniotic membrane (AM). Nine patients were suffering from chemical burns, five from other conditions. Expression of epithelial-lineage markers (keratin 3 [K3], K12, K19 and MUC5AC), inflammatory cytokines (IL-1α and -1β), adhesion molecules (ICAM-1 and VCAM-1) and VEGF was analyzed using real-time PCR and western blotting of the pannus excised during SC transplantation. Healthy human cornea (n = 6) and conjunctiva (n = 6) served as control tissue. A total of 6 months after SC transplantation, an additional penetrating corneal transplantation was performed in two patients due to deep scarring and vascularization. Excised corneal buttons were analyzed to assess the effect of SC transplantation.

Limbal SC formed a monolayer of small undifferentiated epithelial cells on AM. These cells had low expression of differentiation markers K3 and Cx43 but high expression of potential SC markers such as ABCG2 and p63.

The expression of all studied markers differed significantly in healthy cornea and conjunctiva. Levels of K19 and MUC5AC were increased, whereas K3 and K12 were reduced in conjunctiva compared with cornea. Expression of ICAM-1, VCAM-1, IL-1β and VEGF was higher in conjunctiva than in corneal tissue.

Expression of lineage markers was in pannus similar to conjunctiva, not to cornea. Inflammatory markers such as IL-1β, ICAM-1 and VCAM-1 were significantly increased in pannus compared with normal corneal or conjunctival tissue. IL-1α was only moderately increased in chemical burn patients, but higher in the other patients. VEGF was expressed to a greater extent in pannus, when compared with cornea.

Generally, SC transplantation improved vision and reduced inflammation, vascularisation and discomfort. Six months after SC transplantation lineage markers showed a characteristic corneal phenotype. The expression of inflammatory markers was significantly reduced.

In conclusion, LSD is characterized by an abnormal inflamed tissue with conjunctival phenotype. Transplantation of *in vitro* cultivated limbal SCs on AM improved vision significantly, preserved corneal epithelial phenotype and facilitated restoration of a non-inflamed corneal ocular surface.

60.

Stem cell therapy in chronic cardiac failure

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Keywords: chronic cardiac failure, stem cell

Heart failure is a dangerous disease with an increasing frequency. Although conventional drug therapy may delay remodeling, there is no basic therapeutic regime available for preventing or even reversing this process. Heart failure impairs the quality of life and increases expenses to a great extent. Several preclinical, as well as clinical, trials have shown that transplantation of autologous bone marrow cells or precursor cells improved cardiac function after myocardial infarction and chronic heart disease. Due to the fact that transplantation of mononuclear cells is an approved and safe method, we investigated the effects of intracoronary autologous stem cell transplantation in patients with non-ischemic dilated cardiomyopathy.

Methods

A total of 16 patients with dilated cardiopmyopathy (group 1) and 18 patients with chronic myocardial infarction aged 5 months to 8.5 years old (group 2) were included in this study. The control group also consisted of 16–18 age- and sex-matched patients with comparable ejection fractions. Coronary artery disease and myocarditis were excluded in group 1 by coronary angiography and endomyocardial biopsy. All patients of both groups received intracoronary autologous mononucelar stem cells. All the details of stem cell transplantation and invasive procedures have been published previously in detail. All patients had cardiac failure of severity degree New York Heart Association (NYHA) II–IV.

Results

A total of 3 months after intracoronary cell therapy, the global left ventricular ejection fraction increased in group 1 from 18 ± 1 to 23 ± 3%. In parallel, the physical ability (functional capacity) rose from 25 W to 75 W. In addition we found an improvement of maximum oxygen uptake under stress from 1236 ± 217 ml/min to 1473 ± 198 ml/min. Furthermore we documented a reduction of arrhythmias. An unchanged, or even impaired, left ventricular function was not observed in any patients from group 1. In the control group, no significant changes were documented. No side effects of intracoronary autologous stem cell therapy were found, particularly no arrhythmias, no heart insufficiency, no dyspnea and no palpitations. In group 2, after 3 months, infarct size was reduced by 30%, global left ventricular ejection fraction improved by 15% and infarction wall movement velocity improved by 57%. All changes reached a significant level, whereas in the control group no significant changes were observed concerning infarct size, left ventricular ejection fraction or wall movement velocity of the infarcted area. Percutaneous transluminal coronary angioplasty alone had no effect on left ventricular function. After bone marrow cell transplantation, there was an improvement of maximum oxygen uptake (VO_{2max}) (+11%) and of regional 18F-fluor-desoxy-glucose uptake into infarcted tissue (+15%).

Conclusion

These results demonstrate that functional and metabolic myocardial regeneration in patients with dilated cardiomyopathy (group 1) and in patients with chronically infarcted tissue (group 2) can be realized by bone marrow mononuclear cell transplantation.

61.

In vivo reconstruction of adult neuronal projections by human embryonic stem-cell derived neural stem cell transplantation

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Keywords: axon outgrowth, human embryonic stem cells, neural stem cells

The potential of neural grafts to replace adult brain tissue in a structurally and functionally relevant way is an essential prerequisite for reconstructive approaches. So far most emphasis was put on the derivation of desired cell types from various sources and the assessment of their ability to survive and differentiate *in vivo*, but the capability of donor cells to grow axons and dendrites in a meaningful way in the adult brain is foreseen with skepticism.

To evaluate the potential of recently generated human embryonic stem cell-derived neural stem cells (hES-NSCs) to differentiate and grow projections in the adult brain we grafted them into the primary motor cortex as well as into the hippocampus of rodents. Potent neuronal differentiation of the graft was followed up to 1 year. Most donor neurons acquired an inhibitory (GAD^+) phenotype; excitatory neurons ($vGlut2^+$) were in the minority. Markers of immature cells or proliferation were not detectable after 6 months, demonstrating the safety of the grafts.

Human neurons showed a projection profile comparable to that of endogenous neurons in the transplant area.

Donor cells could be targeted stereotactically to the dentate gyrus where endogenous cells degenerated in response to the transplantation. Human axons projected preferentially to the ipsilateral CA3 region where xenogenic synapses were identified, thereby reconstructing the partly degenerated mossy fiber pathway. From the CA3 region human axons entered the fimbria, crossed to the contralateral hemisphere and made contact specifically to the contralateral hippocampus.

Motor cortex transplants sent their axons into the capsula externa from where they re-entered the cortex on the contralateral side, resembling commissural neurons. Subsets of human axons originating from the motor cortex site entered the capsula interna, and could as well be identified in the basal ganglia.

Since hES-NSCs were shown to survive, differentiate safely and, most importantly, adopt region-specific projection patterns we propose that they are to date the best available cell source for nervous system repair.

62.

Mesenchymal condensation in 3D culture

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Keywords: 3D culture, mesenchymal condensation, mesenchymal stem cells

The identification and characterization of progenitor cells during the different stages of mesenchymal differentiation depending on the state of their surrounding microenvironment is the prerequisite for any future strategy in therapeutic regenerative interventions. Genetic defects at the stage of cell condensation lead to embryonic lethality and are, therefore, difficult to analyze in humans or in the murine system. Therefore, a modified culture system was developed that mimics the process *in vitro* and may finally help to identify the key factors that are essential for the induction of mesenchymal differentiation *in vivo*.

Aiming at the identification of key factors that initiate a directed differentiation we study the stages of such a process starting with *in vitro* expanded mesenchymal cells isolated from cartilage biopsies and fat tissue. During the expansion the cells dedifferentiate to progenitor-like cells marked by an expression of mesenchymal stem cell surface markers (CD105, CD73, CD90, CD44, CD106 and CD13, negative for CD34 and CD45) and the capability to differentiate along several mesenchymal linages.

Encapsulation of the cells in alginate hollow spheres represents a special 3D culture system where different phases of cell aggregation starting from a single-cell suspension can be analyzed. After encapsulation, the cells start to accumulate and finally form high-density aggregates. The cells stop in proliferation and are marked by the expression of transcription factors

and SOX-9 SOX-5 and the adhesion molecule N-cadherin followed by the differentiation along the chondrogenic lineage evidenced by a strong induction of collagen II and proteoglycan expression. Similar experiments were conducted with bone marrow derived mesenchymal stem cell. Genome wide expression analysis by microarray technology revealed an altered expression of genes involved in cell cycle regulation resulting in cell cycle arrest as prerequisite of differentiation. Furthermore we observed several growth factor signaling pathways to be affected. Since we do not treat the cells with any stimulating growth factors so far (usually done in differentiation assays) a differentiation to other cell types of the mesenchymal lineage can not be excluded. Exploitation of this new 3D culture system will be helpful to elucidate which genes and environmental factors are involved in early stages of mesenchymal differentiation.

63.

Contribution of Stat5 to the regenerative capacity of mammary epithelial stem cells

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Keywords: constitutively active Stat5, mammary stem cells, short hairpin Stat5, signal transducer and activator of transcription 5

The mouse mammary gland represents a unique model for the study of growth, differentiation and the control of organogenesis. It consists of branching epithelial ducts that are embedded in the fat pad displaying functional differentiation during pregnancy and lactation [1]. The transplantation of isolated mammary epithelial cells into cleared fat pads results in the formation of ductal trees and functional alveoli [2]. The focus of our investigation is the contribution of individual genes to the functional development of the epithelial compartment in this organ. The signal transducer and activator of transcription 5 (Stat5) is a key regulatory transcription factor in mammary gland development and differentiation [3]. However, the contribution of Stat5 for the self-renewal capacity and the repopulation potential of mammary epithelial cells and its role in breast cancer remains poorly understood.

Freshly isolated mouse mammary epithelial cells were genetically modified by transduction with lentiviral gene-transfer vectors encoding a Stat5 specific small hairpin RNA (shStat5) or a constitutively active variant of Stat5 (cS5F). This mutant is constitutively phosphorylated on Y964 and activated in the absence of inducing cytokine signals [4]. This approach allowed us to target a small fraction of stem cells within the total population of isolated mammary epithelial cells. The transduced cells were transplanted into the mammary fat pads of 3-weeks old mice cleared of endogenous epithelium. The consequences of the downregulation or upregulation of Stat5 in mammary epithelial stem cells on their repopulation capacity and functional differentiation was assessed in virgin and pregnant mice.

Stat5-deficient cells gave rise to ductal structures, similar to the ones observed in virgin mice, but appeared narrower with less branching. More pronounced differences were observed during pregnancy, where Stat5 deficient cells failed to undergo lobulo-alveolar differentiation. Conversely, the upregulation of Stat5 caused a dramatic thickening of the primary ducts in virgin mice and precocious development of alveoli.

In conclusion, we have shown that the lentiviral vector mediated loss of function and gain of function of specific genes in mammary stem cells permits rapid and efficient analysis of gene function in mammary gland development.

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64.

Stem cell therapy in patients with chronic coronary artery disease and implantable cardioverter defibrillator is feasible and triggers no arrhythmias

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Keywords: arrhythmia, coronary artery disease, stem cells

Remodeling of the left ventricle (LV) after myocardial infarction (MI) represents a major cause of infarct-related heart failure, death and serious arrhythmias. Despite immediate use of pharmacotherapeutics and mechanical interventions, the vast majority of cardiomyocytes lost during MI cannot be regenerated.

Clinical studies suggest that stem cell transplantation is feasible and has the potential for beneficial effects concerning function, perfusion and metabolism of chronic infarcted hearts. However, especially with skeletal myoblast transplantation, serious arrhythmias have been monitored and the arrhythmogenic potential of peripheral or bone marrow derived stem cells is discussed controversially.

Here we report the first results of holter analysis after stem cell transplantation in patients with chronic myocardial infarction and preexisting implantable cardioverter defibrillator (ICD).

Hypothesis

We assessed the hypothesis that after transplantation of autologous bone marrow derived stem cells the frequency of serious arrhythmias, which are monitored by a preexisting ICD, remains stable or declines.

Methods

Eight patients with chronic myocardial infarction (ages 4–34 years) were transplanted intracoronarily with autologous, mononuclear BMCs. All patients had a pre-existing ICD, which have been implanted according to the recommendations of international guidelines.

Bone marrow was harvested from the hip (~40 ml) and mononuclear cells were identified including CD34 and AC133 positive cells. The intracoronary cell transplantation was performed by using four to five fractional infusions over 2–4 min of 4–5 ml cell suspension. Cells were infused into the necrotic zone through the infarct-related artery via an angioplasty balloon catheter which was inflated to prevent back stream of cells and produce stop flow beyond the site of balloon inflation.

ICD-Holter was analyzed every 3 months before and after stem cell transplantation.

Results & discussion

The follow up period after cell transplantation ranges from 3 months to 20 months (8 ± 6 months). Before stem cell transplantation the ICD-Holter recorded no ventricular arrhythmias in 4 patients. Afterwards there have been no ventricular arrhythmias either. All four patients received β-blockers as standard therapy.

The other four patients showed heterogenous results:

Patient A received three shocks 1 month before transplantation and three shocks 15 months after transplantation. Amiodarone was added to his medication.

Patient B: The holter recorded 87 non sustained ventricular tachycardias and one successful burst during 12 months before and five nonsustained ventricular tachycardias during 10 months after transplantation.

Patient C: The holter recorded two nonsustained ventricular tachycardias before and no ventricular episode after transplantation.

Patient D: He received one adequate shock due to ventricular flutter one month before transplantation. No ventricular episodes have been recorded during 7 months after transplantation.

Conclusion

The data show that, under clinical conditions, autologous bone marrow derived stem cell transplantation is safe and seems to have no arrhythmogenic potential. Moreover, despite the small sample size, stem cell therapy may have a beneficial effect concerning the appearance of serious ventricular arrhythmias.

Cancer & stem cells

66.

Absence of Oct4 expression in somatic tumor cell lines

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Keywords: Oct4, somatic tumor cells

The POU-domain transcription factor Oct4 is associated with the pluripotent state of cells comprising the inner cell mass of pre-implantation embryos and plays a critical role in the maintenance of pluripotency of embryonic stem cells derived from early embryos. By contrast, reactivation of Oct4 expression has been discussed to occur also in differentiated cells that have undergone carcinogenesis, or tumor formation. Two recent reports describe that the cervical carcinoma cell line HeLa and the breast cancer cell line MCF7 express Oct4, which is in striking contrast to earlier studies. To address this discrepancy we determined Oct4 expression in these human tumor cells lines in comparison with the human teratoma cell line nTera by immunofluorescence, western blot, and RT-PCR analyzes. We were unable to detect staining of the Oct4 transcription factor in the nucleus of HeLa and MCF7 cells by immunofluorescence using two different monoclonal antibodies. Although a very faint cytoplasmic Oct4 protein staining in HeLa and MCF7 cells was observed, an Oct4 signal could not be detected by Western blot analysis. In addition, we were unable to detect Oct4 mRNA in HeLa and MCF7 cells by RT-PCR. We argue that recent reports of Oct4 expression in these and other cancer cell lines may be due to Oct4 pseudogene expression or misinterpretation of background signals in immunofluorescence. We conclude that neither HeLa nor MCF7 cells express Oct4 under standard cell culture conditions and reinforce the need for adequate controls in immunofluorescence and RT-PCR analyzes.

67.

Characterization of Musashi-1 expression in human endometrium: possible implications for the pathogenesis of endometriosis and endometrial carcinoma

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Keywords: endometrial carcinoma, endometriosis, endometrium, musashi-1, Notch, progenitor cell, telomerase

The human endometrium undergoes cyclical processes of regeneration, differentiation and shedding during the menstrual cycle. It has been proposed that progenitor cells are responsible for this remarkable regenerative capacity, and may contribute to the pathology of proliferative diseases such as endometriosis and endometrial carcinoma. However, endometrial progenitor cell populations are presently only poorly characterized, establishing a need for molecular markers of endometrial stem cells. The RNA-binding protein Musashi-1 (Msi-1) is associated with maintenance and asymmetric cell division of neural and epithelial progenitor cells, acting via modulation of the notch-1 signaling pathway. Using immunohistochemistry, quantitative real-time PCR and immunofluorescence microscopy, expression and localization of Msi-1 was investigated in endometrial, endometriotic and endometrial carcinoma tissue specimens of 46 patients to characterize a potential contribution of Msi-1 to these pathologies. Quantitative real-time PCR analysis confirmed Msi-1 expression in human endometrium. Expression was significantly lower in myometrium as compared with endometrium. Immunofluorescence microscopy revealed a coexpression of Msi-1 and its molecular target notch-1. Moreover, Msi-1 positive cells coexpressed the catalytic subunit of telomerase (hTERT). The number of Msi-1-expressing cell groups/high power microscopic field was significantly increased by approximately fourfold during the proliferative phase in comparison with the secretory phase. Of note, the number of Msi-1 expressing stromal cells and cell groups/high power microscopic field was significantly increased by approximately 5–8-fold in endometriotic tissue compared with secretory endometrium of control subjects. Similarly, the number of Msi-1 positive stromal cells was significantly increased approximately fivefold in endometrial carcinoma compared with secretory endometrium of control subjects. In summary, we have demonstrated the presence of a putative endometrial progenitor cell population expressing the neuronal/epithelial stem cell marker Msi-1. The demonstration of a coexpression of Msi-1 and hTERT underlines the progenitor cell character of Msi-1 positive cells. Our finding of a significantly increased number of Msi-1 expressing progenitor cells in endometriotic and endometrial carcinoma tissues compared with secretory endometrium supports the emerging concept of a stem cell origin of these diseases.

68.

Maintenance of genomic stability in breast cancer stem cells: role of reactive oxygen species and H2AX phosphorylation

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Keywords: breast cancer stem cells, DNA repair

The etiology of breast cancer is still poorly understood. The observation that breast cancer recurrences can occur more than 10 years after primary surgery suggests that a rare subpopulation of cancer cells, cancer stem cells, may be the driving force behind tumorigenesis. The central characteristics of stem cells are their capacity for unlimited self-renewal and the retention of pluripotency to differentiate. While the mechanisms underlying stem cell differentiation have been well examined, less is known regarding how stem cells avoid cell death and resist genetic damage. Since normal adult stem cells are slowly dividing, long-lived cells and the precursors to differentiated cells, DNA repair and mutation avoidance in these cells should be critical.

The aim of this study is to identify and characterize putative cancer stem cell genes that correlate with the ability to avoid genotoxic agents and to determine their role in tumor development and progression. We therefore analyzed a panel of DNA repair proteins in a population of breast cancer stem cells derived from the MCF7 breast carcinoma cell line. We also looked at the accumulation of reactive oxygen species, DNA damage and repair in single cells, H2AX phosphorylation, and telomerase activity. Our results show that the stem cells have markedly higher telomerase activity, and accumulate significantly lower amounts of reactive oxygen species and H2AX. Thus, compared with cancer cells, cancer stem cells have developed an extensive defense system that contributes to their ability to survive in the presence endogenous and exogenous agents.

69.

Identification and characterization of the adrenal tumor side population

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Keywords: adrenal, adrenocortical tumor, cancer stem cell, side population

Recent evidence suggests the existence of a stem cell-like subpopulation of cells in hematological and solid tumor entities, which determine the malignant phenotype of a given tumor through their proliferative potential and chemotherapy resistance. A recently utilized technique for the isolation of this cell population is through exclusion of the vital dye Hoechst 33342, which defines the so-called side population (SP). Herein we demonstrate the presence of SP cells in a variety of adrenal specimens, including primary cultures of human adrenocortical tumors and normal adrenal glands as well as established human and murine adrenocortical cancer cell lines by fluorescence activated cell sorting analysis and confocal microscopy. On a functional level, SP cells from the human adrenocortical tumor cell line NCI h295R revealed an expression pattern consistent with a less differentiated phenotype, including lower expression of steroidogenic enzymes such as steroid acute regulatory protein and side chain cleavage enzyme (P450scc) in comparison to non-SP cells. However, SP and non-SP cells demonstrated the same proliferative potential (105.6 ± 18.1% vs 100.0 ± 3.5%), and both cell types gave rise to the original SP and non-SP containing cell population in resorting and tracing experiments over three passages. In addition, no survival benefit for SP cells was evident after treatment with cytotoxic agents commonly used in adrenocortical carcinomas. Taken together, in the adrenocortical tumor cell line NCI h295R, the Hoechst dye exclusion phenomenon, in contrast to other primary tumors or tumor cell lines, does not represent a major tumor stem cell defining marker.

70.

TCam-2 but not JKT-1 resemble seminoma in cell culture and might be an *in vitro* model for primordial germ cells

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Keywords: cell line, germ cell tumor, gonocyte, neoplastic, seminoma

A total of 60% of all malignancies diagnosed in men between 17–45 years of age are germ cell tumors (GCTs). GCTs arise from carcinoma *in situ* cells, which are thought to originate from a transformed fetal germ cell, the gonocyte. Seminoma together with embryonal carcinoma represent the most frequent subtypes of GCT. However, the nature of the molecular pathways involved in seminoma formation remains elusive. Therefore, analysis of appropriate cell culture systems is an important prerequisite in further understanding of the etiology of this tumor entity. While several cell lines for embryonal carcinoma have been established and analyzed, so far only two cell lines from seminoma patients have been reported. In the present study, we analyzed these seminoma cell lines, TCam-2 and JKT-1, and compared the gene expression profiles to normal tissue as well as seminoma and embryonal carcinoma using DNA Array technology. We found that TCam-2 clusters with the group of classical seminoma, whereas JKT-1 clusters to the group of embryonal carcinoma. Using RT-PCR, western blot

and immunohistochemistry we confirmed the seminoma-like nature of TCam-2, whereas JKT-1 lacks expression of most of the genes detectable in GCTs questioning the germ cell nature of this cell line. The data represent the first genome-wide expression analysis of the two cell lines and comparison/clustering to subgroups of germ cell tumors. Hence, only TCam-2 represents a suitable *in vitro* model for seminoma.

Furthermore TCam-2 might be an adequate *in vitro* model for primordial germ cell studies, due to the expression of characteristic markers such as BLIMP1, PRMT5 and pluripotency markers such as Oct3/4 and Nanog, as well as KIT and AP-2ã. In addition, an arginine dimethylation of histone H2a and H4 could be detected by Western blot in TCam-2. This dimethylation is a consequence of the BLIMP1/PRMT5 comlex co-localization in the nucleus and was shown in migrating and undifferentiated cells such as gonocytes and primordial germ cells. So TCam-2 might be a valuable model to recapitule early steps of germ cell development.

71.

In vivo cell tracking via NMR spectroscopy allows online observation of stem cell based therapeutic approaches in a rat glioma model

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Keywords: cell tracking, magnetic resonance tomography, mesenchymal stem cells, micron-sized iron oxide particles

Stem cell-based therapeutic approaches are promising in the cure of diseases that are resistant to conventional interventions such as malignant brain tumors as a prominent and significant challenge. With the immunologic barrier and formation of teratomas still to overcome, as well as ethical concerns, embryonic stem cells are not suitable for clinical applications yet. On the other hand adult mesenchymal stem cells are easily available from autologous cell preparation and formation of teratomas has never been reported so far. Moreover, they exhibit the potential to adopt a neural phenotype, which enables long-term integration even within the CNS. Nevertheless, concerning efficacy and compliance of cell-based therapies the distribution of the injected cells needs to be observed *in vivo* on an individual basis. Magnetic resonance tomography is a noninvasive technique allowing multiple measurements with high frequency, but the contrast agents available so far require very high labelling concentrations, affecting cell viability, although exhibiting a detection limit of 1000 cells on a spot. Here we present a labelling method using polystyrene-coated iron microspheres with an effective concentration not affecting cell viability or migratory potential and a detection limit of approximately ten cells on a spot. Migration of labeledmesenchymal stem cells was successfully tracked after injection into the brain using a rat glioma model, hence providing a promising tool for online observation of cell-based therapeutic approaches.

72.

Cell fusion: the origin of cancer stem cells?

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Keywords: breast cancer, cancer stem cells, cell fusion, hybrid cells

Cell fusion is a cell-biological phenomenon, which was originally described as an event of development and homeostasis. Moreover, recent discoveries give rise to the theory that fusion events between stem cells and tumor cells could act as malignant and crucial originators in cancer stem cell (CSC) generation, besides other theories such as CSCs deriving from tissue stem cells that have accumulated genetic aberrations or CSCs deriving from differentiated progenitor cells that have regained self- renewing capacity. By now CSCs are known as cancer-initiating cells with characteristic features of stem cells, such as self-renewing, differentiation, regeneration of tissues at low cell numbers and drug resistance. This rare population of cancer-initiating cells attracts attention as a target in cancer therapies and, therefore, there is an urgent need for further investigation on CSCs is present. After 24 h co-cultivation of M13SV1 enhanced green fluorescent protein (EGFP)-neo breast stem cells and invasive HS578T-Hyg breast cancer cells under selective conditions, cell clones that emerged from spontaneous fusion events were isolated and cultivated separately. Short tandem repeat analysis of hybrid cells showed an overlap of the parental alleles, indicating that hybrid cells have been originated from real cell fusion events.

Despite some varieties concerning the data of the further hybrid cell characterization due to different genetic profile among them, one can detect clear tendencies of similarity between all hybrid cell lines compared with their parental cell lines. XTT-proliferation assays, for example, show a significantly higher proliferation rate of the hybrid cell lines compared with their parental cell lines. Dependent on the hybrid cell line a 5–10-times higher proliferation rate could be detected in comparison with the breast stem cell line M13SV1 EGFP-neo, whereas, in contrast to the breast cancer cell line HS578T Hyg, the proliferation shows an up to four-times higher rate. Moreover, expression pattern analysis by real-time PCR data revealed interesting results in multiple up- and down-regulation of cancer and drug metabolism genes compared with the HS578T cell line and the M13SV1 cell line, for example, upregulation of cell cycle regulators as p16 and p53 in all hybrids, drug resistance transporters as ABCC6, ABCB1 and major vault protein (MVP) in all hybrid cell lines as well as upregulation of androgen receptor in three of four hybrid cell lines, which stands in a reciprocal relationship to downregulation of estrogen receptor in some poor prognosis breast cancers, probably indicating that fusion might cause a switch to an estrogen-independent tumor growth. The real-time PCR data have been confirmed by Western blot analysis. On the basis of spontaneous fusion between M13SV1 breast stem cells and invasive HS578T breast cancer cells we could give a promising insight into characterization of fusion cells as a possible model for CSCs.

73.

In vitro analysis of CD133/prominin-1 following lentiviral transfection in established human glioma cell lines

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Keywords: CD133/prominin-1, glioma, tumor stem cells

CD133 or prominin-1 is a transmembrane protein that is expressed by several types of somatic stem cells, including neural and hematopoietic stem cells. Furthermore, CD133 was reported as a promising marker for tumor stem cells in primary glial brain tumors (Singh *et al.*, *Nature.* 432, 396–401 2004). Here, we investigated the expression of CD133 transcripts in a series of 95 astrocytic brain tumors by using real-time RT-PCR analysis. Thereby, we found variable expression of CD133 at the mRNA level in the majority of gliomas, with the highest expression levels being present in the most malignant tumors, in other words, the glioblastomas. In contrast to the results obtained in primary tumors, established glioblastoma cell lines grown in monolayer cultures under standard conditions completely lacked detectable expression of CD133 mRNA and protein. As the function of CD133 in glioma cells remains unknown, we investigated the effects of ectopic CD133 expression in established glioma cell lines (T98G, U138MG) after lentiviral transduction. CD133 expression in stably transfected cell lines was validated by flow cytometry, immunofluorescense staining and real-time RT-PCR. So far, investigations of the influence of ectopic CD133 expression on cell proliferation, vitality and apoptosis of glioma cells *in vitro* did not reveal significant differences between CD133-transduced versus control (GFP)-transduced cells. Additional *in vitro* experiments as well as microarray-based expression profiling studies are currently under way to provide clues for the functional role of CD133 in glioma cells.

74.

Human periodontium-derived neural stem cells: chance or risk?

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Keywords: adult stem cell, cancer stem cell, minimally invasive adult stem cell isolation, periodontal stem cell, serum-free culture

Neural stem cells (NSCs) are potential sources for cell therapy of neurodegenerative diseases and for drug screening. Despite their potential benefits, ethical and practical considerations limit the application of NSCs derived from human embryonic stem cells or adult brain tissue. Thus, alternative sources are required to satisfy the criteria of ready accessibility, rapid expansion in chemically defined media and reliable induction to a neuronal fate. We isolated somatic stem cells from the human periodontium that were collected during minimally invasive periodontal access flap surgery as part of guided tissue regeneration therapy. These cells could be propagated as neurospheresin serum-free medium, which underscores their cranial neural crest cell origin. Culture in the presence of EGF/FGF-2 under serum-free conditions resulted in large numbers of nestin-positive/Sox-2-positive NSCs. These periodontium derived (pd) NSCs are highly proliferative and migrate in response to chemokines that have been described as inducing NSC migration. We used immunocytochemical techniques and RT-PCR analysis to assess neural differentiation after treatment of the expanded cells with a novel induction medium. Adherence to substrate, growth factor deprivation and retinoic acid treatment led to the acquisition of neuronal morphology and stable expression of markers of neuronal differentiation by more than 90% of the cells. Thus, our novel method might provide nearly limitless numbers of neuronal precursors from a readily accessible autologous adult human source, which could be used as a platform for further experimental studies and has potential therapeutic implications.

Surprisingly, transplantation of periodontium-derived stem cells to nude rats resulted in an high incidence of malignant epithelial tumors. Presence of human mitochondria antigens suggests a tumor formation by the transplanted cells. Analysis of the karyotypes of pdNSCs showed a high degree of aneuploidity, with chromosome counts peaking at 70 chromosomes. Taken together, these data might suggest that culture of pdNSC could have induced aneuploidity, which might be a reason for tumor formation. Thus, the development of therapy might consider optimization of culture conditions to avoid polyploidity.

Stem cells & aging

76.

Adipose-derived marrow stromal cells from lipoaspirate are CD271-positive

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Keywords: adipose tissue, CD271, low-affinity nerve growth factor receptor, MACS®, marrow stromal cell, miltenyi biotec, stem cell

Marrow stromal cells (MSCs) are receiving more and more focus for a variety of clinical research applications, such as for the reduction of graft-versus-host disease after allogeneic transplantation, or the treatment of osteogenesis imperfecta and metabolic diseases. Bone marrow has long been known to be a rich source for MSCs. However, over the last few years, alternative sources in humans have also been shown to contain MSC-like cells, such as adipose tissue, placenta and umbilical cord blood6. With a higher colony forming unit fibroblasts (CFU-F) activity compared with bone marrow, and the ability to differentiate into cells of the mesenchymal lineage *in vitro*, adipose tissue comprises a source of stem cells that can be obtained under less invasive conditions for the patient than bone marrow aspiration. These stem cells are referred to as adipose-derived mesenchymal stem cells (AMSCs). Adipose tissue, in the form of cosmetic lipoaspirate, comprises a heterogeneous cell mixture composed of, among others, endothelial cells, hematopoietic cells, smooth muscle cells, pericytes and cells of mesenchymal origin. Several researchers have shown that MSCs from bone marrow can be enriched very effectively by selection for CD271 expression and have already been used successfully in animal studies. It is also thought that AMSCs are CD271 positive. Here, we demonstrate that CD271 can be used to highly enrich AMSCs from lipoaspirate.

Method

Aspirate was processed using a standard protocol. The stromal vascular fraction was analyzed by flow cytometry using the following antibodies: CD14, CD34, CD45, CD90, CD105, CD146, CD73 and CD271. CD271 enrichment was performed using the CD271 (low-affinity nerve growth factor receptor) MicroBead Kit (APC), containing CD271 (low-affinity nerve growth factor receptor)-APC and Anti-APC MicroBeads, and NH Expansion Medium was used to expand CD271-positive cells. The phenotype and CFU-F activity of CD271-isolated cells was compared with that of AMSCs isolated by plastic adherence under the same cultivation conditions. Furthermore, the differentiation potential of isolated cells into adipocytes (NH AdipoDiff Medium), chondrocytes (NH ChondroDiff Medium), and osteoblasts (NH OsteoDiff Medium) was investigated.

Results

The stromal vascular fraction contains a population of $CD271^{+}CD45^{-}$ cells (18%), which could be enriched to a purity of approximately 81% (n = 4). By comparing the CFU-F activity of unseparated stromal vascular fraction with that of the CD271-negative and -positive cell fractions, it was found that the CD271-selected fraction contained the highest number of colonies. Regardless of the isolation method, all AMSCs obtained showed a similar potential to differentiate into adipocytes, chondrocytes, and osteoblasts.

Conclusion

Our results show that MSCs from adipose tissue as well as bone marrow are CD271-positive, can be highly enriched using MACS® Technology, and maintain the ability to differentiate to cells of adipocyte, chondrocyte and osteoblast lineages.

77.

Potential aging effects in long-term cultured mouse neurospheres

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Keywords: aging, neurospheres

Neural cells are isolated from forebrain of 14.5 days old mouse embryos. In selective conditions these cells cluster forming sphere-like structures – neurospheres (NSCs). Neurospheres are heterogeneous structures containing only 2–5% of stem cells and progenitors being reportedly capable to transdifferentiate. By some authors, reproducibility of such experiments was poor but the age of transplanted NSCs was not considered one of the factors potentially responsible. As a matter of fact, it is generally assumed that progenitors and stem cells remain intact in long-term culture. Based on this notion, long-term progenitor cell cultures are supposed to be unaltered and convenient for potential transplantation. Despite this, we hypothesized that potential aging effects of overall sphere certainly will impact the fraction of stem cells and progenitors. Therefore, this assumption was tested exploring distinct aspects of aging in long-term NSC culture.

Potential alterations that might occur due to aging were monitored within 1–16 weeks of culturing. Initially, tremendous structural and numerous chromosomal aberrations were observed upon 16 weeks of culturing. This was accompanied with p53 up-regulation. Moreover, p53 engagement may indicate higher apoptotic rate as a self-defence mechanism in order to prevent further DNA burden. Increasing appearance of chromosomal aberrations might be the consequence of aging and a signal strong enough to induce senescence. Telomere shortening was analyzed as an indication of 'senescent mitotic clock' what may imply reduced number of cell divisions from a certain time point. Preliminary telomere measurement indicated decrease in telomere length after 5 and 16 weeks. Although with jeopardized overall homeostasis, NSC unexpectedly displayed gradually elevated capacity to form spheres even in seeded low cell density up to 16 weeks culturing. Increased capacity to form spheres is accompanied with decreasing ability to differentiate into neural lineages.

Genetic instability and diminished differentiation capacity seem to be a consequence of long-term culturing implying potential transformation. The reduced differentiation potential after long-term culturing might be the reason for the low reproducibility of transdifferentiation experiments.

Bioengineering & biomaterials

78.

Human mesenchymal stem cells: *in vitro* proliferation and chondrogenic differentiation in fibrin glue

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Keywords: chondrogenic differentiation, fibrin glue, stem cells, tissue engineering

Recently, tissue engineering has merged with stem cell technology to develop new sources of transplantable material for injury or disease treatment. Major long-term goals are tissue and organ replacement therapies using autologous cells. Owing to the limited availability of autologous chondrocytes, for appropriate therapies, the use of adult human stem cells represents a promising option.

The purpose of this study was to create a matrix, which provides a suitable scaffold for reimplanting cells, minimizing necrotic areas and maintaining cell-proliferation as well as their capacity to develop a chondrogeneic character. Therefore, pluripotent stem cells, isolated from adult human bone marrow (human mesenchymal stem cells [hMSCs]), were integrated into fibrin sealant (Tissucol and Baxter), composed of thrombin and fibrinogen. They were cultured *in vitro* throughout a period of 21 days either in proliferation medium or under conditions of chondrogenic differentiation. Applying centrifugal forces, we were able to create a large-porous fibrin matrix, allowing hMSCs to integrate and survive throughout the period of culture. In order to closer emulate the poor blood supply of cartilage tissue, besides normoxic conditions (20% O_2), hypoxic conditions (3% O_2) were used for culturing. The hMSCs integrated into the fibrin matrix were analyzed with regard to morphology, proliferation and pluripotency, especially concerning their capacity to adopt a chondrogeneic character.

Morphology and proliferation of the embedded hMSCs did not markedly vary concerning normoxic and hypoxic culture conditions, but noteworthy proliferation could still be detected after 21 days in culture, revealed by staining with anti-Ki67 antibody. In addition, we observed rounded chondrocyte-like cell types with an ovoid nucleus in the fibrin construct cultured under differentiation. Using RT-PCR we could show that the hMSCs retain their pluripotent character through expression of transcription factor Oct-4 during the whole culture period. Furthermore, under conditions of chondrogenic differentiation, we were able to detect a gradient chondral phenotype assessed by mRNA expression of collagen II, which was confirmed *in situ* by means of alcian-blue staining. Chondrogeneic differentiation occurred even under normoxia but most notably under hypoxic conditions.

In summary, hMSCs seeded into large-porous preparations of the biomaterial Fibrin (Tissucol®) stay alive, proliferate and keep their stem cell character. Furthermore, culturing in corresponding medium induces chondrogeneic differentiation, remarkably enhanced under hypoxic conditions, pointing towards the benefit of emulating the poor blood supply *in vivo* concerning chondrogenesis. Finally, this carrier matrix is applicative for autologous cartilage transplant construction *in vitro* and this study supports the fact that in combination with hMSCs this tissue engineering approach may have clinical applications, at least in reconstructive surgery of cartilage.

79.

Assessment of stem cell/biomaterial combinations for tissue engineering applications

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Keywords: high-throughput screening, polymers, tissue engineering

Biomaterials are used in tissue engineering with the aim of repairing or reconstructing tissues and organs. Frequently, the identification and development of biomaterials is an iterative process, with biomaterials being designed and then individually tested for their properties in combination with one specific cell type. However, recent efforts have been devoted to systematic, combinatorial and parallel approaches to identify biomaterials suitable for specific applications. Embryonic and adult stem cells represent an ideal cell source for tissue engineering. Since stem cells can be readily isolated, expanded and transplanted, their application in cell-based therapies has become a major focus of research. Biomaterials can potentially influence, for example, stem cell proliferation and differentiation in both positive or negative ways, and biomaterial characteristics have been applied to repel or attract stem cells in a niche-like microenvironment. So far, no general principles are known that allow a prediction of the extent of cellular behavior on a given biomaterial. Therefore, cell adhesion, morphology, vitality, proliferation, cytotoxicity and apoptosis have to be analyzed and matched into a basic assessment.

Methods

Cytotoxicity assays according to ISO 10993-5 were performed using FDA/PI staining. Subsequently, stem cells were stained with haemalaun to analyze cell morphology. Next, multiplex assays (Promega kits) were applied for detecting metabolic activity over time, cellular LDH secretion and apoptosis on the polymers.

Results

We introduce a grid-based platform for the assessment of stem cell–biomaterial interactions. We established a biomaterial bank and report the systematic screening of 140 different combinations of stem cells and polymers, demonstrating the usefulness of multifactorial analyzes in the testing of cell–material combinations. We can suggest, for example, the following combinations for tissue engineering applications: human dental pulp stem cells on PDLLA (R203S); human endothelial progenitor cells on PVDF, PTFE, Texin, PLLA (L209S), PLLA-co-PDLLA (Resomer LR705), PEO10-b-PDLLA25 and collagen; human preadipocytes on texin; mouse mesenchymal stem cells on PVDF and texin; and mouse embryonic stem cells on fibrin.

Conclusion

This study demonstrates that the assessment of stem cell–biomaterial combinations for cell-based therapies is multifactorial. Besides material and cell characteristics, cell–biomaterial nteractions have to be investigated. For this, the consideration of one or two parameters only is not sufficient as basic evaluation. In conclusion, stem cell-based therapies using biomaterial scaffolds strictly require a stringent assessment of parameters such as material topography, cell adhesion, morphology, viability, proliferation, cytotoxicity and apoptosis. All these parameters have to be analyzed and matched into a basic assessment.

80.

Laser-assisted strategy for the negative selection of pluripotent human embryonic stem cells from differentiating cultures

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Keywords: cell elimination, cell purification, nanoparticle, neural precursor, pluripotency-associated marker

Due to their pluripotency and their self-renewal capacity, human embryonic stem cells (hESCs) provide fascinating perspectives for biomedical applications. In the long term, hESC-derived tissue-specific cells will constitute an important source for cell replacement therapies in nonregenerative organs. These therapeutic approaches, however, will critically depend on the purity of the *in vitro* differentiated cell populations. In particular, remaining undifferentiated hESCs in a transplant can induce teratoma formation.

In order to address this challenge, we have developed a laser-based method for the ablation of pluripotent hESCs from differentiating cell cultures.

hESCs express tumor-related antigen (Tra)-1-60 and Tra-1-81, which are associated with a pluripotent state of the cells. In a first step, specific antibodies were directed against these surface markers. These antibodies, in turn, were targeted with nanogold particles. Subsequent laser exposure led to the elimination of the labeled cells. Efficiency of the depletion was then determined by flow cytometry and recultivation experiments.

Employing this strategy, we could show that 98.9 ± 0.87% of hESCs were ablated within undifferentiated cell cultures. In order to ensure that cells that express neither of the Tra antigens were not affected by the laser treatment, pluripotent hESCs were mixed with green fluorescent protein-positive neural precursor cells. The elimination efficiency for the target cells within this inhomogeneous cell population remained constant (98.7 ± 1.2%), while no side effects on the neural precursor cells could be observed. These results were confirmed by replating of the cells after laser exposure. Proliferation and differentiation of neural precursor cells did not differ from untreated controls. Most importantly, hESC colony forming units were remarkably reduced with only 0.04 colonies/1000 cells formed after irradiation compared with 25 colonies/1000 cells formed in the untreated control.

Laser-induced, nanoparticle-mediated ablation therefore represents a novel contact-free method for the efficient elimination of contaminating pluripotent hESCs. Since the damage mechanism is based on physical parameters, it can be varied more precisely than chemical methods by choosing appropriate particle size, pulse width and pulse energy. Thus, in combination with a positive selection strategy for the desired somatic cell population, our strategy should contribute to the generation of highly purified hESC-derived tissue-specific cells.

Non-therapeutic applications of stem cells

83.

Transplantation of hematopoietic stem cells transduced with foamyviral vectors in a nonhuman primate model (Callithrix jacchus)

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Keywords: hematopoietic stem cells, foamyvirus, gene transfer, large animal model, nonhuman primate model

Preclinical animal models are important for evaluating the safety and therapeutic efficacy of new therapeutic modalities such as gene therapy. We used common marmosets (*Callithrix jacchus*) because these are small and relatively inexpensive when compared with other nonhuman primate models. Currently, only little information about the efficiency of foamyviral vectors for the transduction of primate stem cells is available. Since foamyviruses are nonpathogenic in their natural hosts, vector systems derived from foamyviruses may be an attractive alternative to lenti- and γ-retrovirus vectors for gene therapy approaches.

Three marmosets were successfully transplanted with transgenic hematopoietic stem cells. Animals were treated with PEGylated granulocyte-colony stimulating factor (G-CSF) 5 days before bone marrow was collected from the femur of anesthetized marmosets. After density centrifugation $CD34^+$ cells were enriched by magnetic separation using the recently cloned monoclonal mouse antimarmoset CD34-antibody MA24. The foamyvirus construct (MH71.MGMT) used for transduction expressed the enhanced green fluorescent protein (EGFP) and O6-methylguanine-DNA methyltransferase (MGMTp140k), separated by an internal ribosome entry site. The genes were expressed from the spleen focus forming virus (SFFV) promoter. MGMTp140K is a drug resistance gene, allowing for *in vivo* selection of transgenic cells. Vectors were pseudotyped with a modified prototype foamy virus envelope. Virus stocks of these vectors were prepared by polyethyleneimine (PEI)-mediated transfection of 293T cells and concentrated approximately fivefold by centrifugation for 2 h at 16,000 g and 4°C. CD34-enriched cells were transduced in 6-well dishes coated with 8 μg/cm^2 CH-296 and in the presence of interleukin-6, FMS-like tyrosine kinase-3, SCF and TPO overnight.

All three animals were transplanted with approximately 4×10^5 $CD34^+$ cells/kg body weight after administration of a mild nonablative conditioning of 1–2 mg/kg Busulfan 24 h prior to transplantation. Hematopoietic recovery was without any complications and the animals are now 31, 21 and 18 weeks post-transplantation. Three weeks after transplantation GFP-positive granulocytes were detectable by flow cytometry. In DNA samples from the peripheral blood GFP could be detected by PCR at time points later than 1 month after transplantation. Peripheral blood cells were also plated in methyl cellulose and GFP-positive cells could be detected 14 days later by fluorescence microscopy.

In conclusion, it is feasible to transduce hematopoietic stem cells with foamyvirus vectors and these cells engrafted successfully after autologous transplantation. These results suggest that foamyviral vectors are an alternative for lentivirus and gammaretrovirus vector systems for stem cell gene transfer and thus set the stage for a more detailed analysis of this vector system in transplantation studies in the nonhuman primate model.

Free Topics

84.

Extracorporal shock wave treatment influences the migration, proliferation and growth of human mesenchymal stem cells

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Keywords: growth, migration, proliferation, shock wave, stem cells

Previously extracorporal shock wave therapy has been established, for example, for the smashing of renal calculi and gallstones. Currently extracorporal shock wave therapy is successfully used for the therapy of pseudoarthrosis, plantar fascitis, tendinosis calcarea and wound healing disorder. Numerous *in vivo* studies underline the good results of this therapy form. In our studies we focus on cellular effects of extracorporal shock wave on migration, proliferation and growth of isolated human mesenchymal stem cells (MSCs). For a therapeutic application of MSCs, different invasive methods have been described previously. Shock waves are generated extracorporally and would represent a first noninvasive way to influence and guide MSCs into the target area. In medicine there are different techniques to generate shock waves. For our studies we used the piezoelectric extension of Piezoson 100 (Wolf Inc.).

Isolated human MSCs were treated with shock waves. Afterwards the migratory activity was analyzed. Our experiments infer that shock wave treatment significantly increased the migratory activity of MSCs up to threefold compared with the untreated controls. Furthermore, the migration of MSCs was strongly influenced by the number of impulses per application (500 or 1000 impulses per application), the intensity level (0.077–0.238 mJ/mm^2) and the frequency of application (two or four per s). In addition, treatment with shockwaves significantly increased proliferation and growth of MSCs.

The shock wave conditions that achieved the best results in migration experiments also resulted in the strongest influence on proliferation and growths of MSCs. The most effective conditions in our studies were four applications per second, 1000 impulses and an intensity level of 0.077 mJ/mm^2.

In conclusion, the strong effects onto the behavior of human MSCs indicate that stem cells can also be influenced against mechanical stimuli like shock waves. The number of impulses per application, the intensity level and the frequency are important parameters for these effects.

85.

Characterization of the interaction between circulating and *in vitro* cultivated endothelial progenitor cells and the endothelial barrier

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Keywords: cell therapy, endothelial barrier, transmigration

In vitro cultured endothelial progenitor cells (cEPC) are used for intracoronary cell therapy in cardiac regeneration. The aim of this study was to investigate whether cEPC and circulating mononuclear cells (MNC), which include a small number of *in vivo* circulating endothelial progenitor cells, are able to transmigrate through the endothelial barrier into the cardiac tissue.

MNC and EPC were isolated from the peripheral blood from healthy male volunteers (n = 13, 25„b6 years (ASK AUTHOR)) and stained with a fluorescent marker. The cells were perfused *in vitro* through organs with endothelial layers of different phenotypes (rat aorta, human umbilical vein, isolated mouse heart). The endothelium and the basal lamina were then stained by immunofluorescence and the cryo-sections analyzed using a confocal laser scanning microscope.

After perfusion through the rat aorta, an adhesion/integration of MNC was observed at the endothelial layer and the basal lamina beneath endothelial cells. However, no migration of MNC over the endothelial barrier was found. This remained true even when the cell numbers were increased (from 0.5 to 10 million cells/h), when the time of perfusion was prolonged (1.5–4 h) and when the aorta was cultivated for 24 h. In the Langendorff-perfused mouse heart with intact endothelium, no migration of MNC (10 × 106 in 0.5 and 2 h) or cEPC (1 × 106 in 0.5 and 2 h) was observed after 0.5 and 2 h.

Conclusions

MNC and cEPC do not possess any capacity to transmigrate the endothelial barrier. In the context of stem cell therapy, these cells may therefore serve as endothelial regenerators but not as cardiomyocyte substitutes.

86.

Establishment of germline stem cells from biopsied testicular tubules of individual mouse

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Keywords: germline stem cells, *in vitro* cluster-formation assay, testis biopsy

Male germline stem cells (GSCs, also called spermatogonial stem cells) are self-renewing cells of the testis that are also capable of differentiating into germ cells. It is possible to isolate GSCs from testis and propagate them in *in vitro* culture over the long term. However, current protocols use whole testes from either neonatal or adult mice, the results of which may not reflect the human scenario. In this study, we attempted to establish a GSC line from biopsied testicular tubules of an individual adult mouse. We biopsied one testis from each of four fertile Oct4-GFP transgenic mice, that had been anesthetized and harvested small samples of tubular tissue. We were successful in establishing three GSC lines from the four biopsied samples. Molecular and cellular characterization confirmed that these cells were indeed of a GSC phenotype. The established GSCs also exhibited cluster-forming activity as assessed by a recently developed *in vitro* cluster-formation assay [1]. Thus, the establishment of GSCs using testicular biopsy in the mouse model has the potential for clinical applicability in humans, providing a method for the derivation of GSCs from individual patients that can be used to treat infertility.

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87.

Purification of PSA-NCAM-positive progenitor cells from the mouse brain and analysis of signal intensity with high resolution magnetic resonance imaging *in vitro* and *in vivo*

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Keywords: immunoselection, ironoxide particles, magnetic cell separation technique, magnetic resonance imaging, polysialic acid-neural cell adhesion molecule

Polysialic acid-neural cell adhesion molecule (PSA-NCAM) is a marker of both immature multipotent neural precursor cells as well as cells that are committed to a neuronal or glial lineage. The polysialylation of NCAM is regulated in the developing embryo, in addition to being present in the adult nervous system. Therefore, it is assumed to play an important role in neural plasticity.

PSA-positive cells were isolated from whole brain and the cortex of embryonic E16 $CD1^-$ and early postnatal mice. The cells were immunoselected using a specific antibody coupled to superparamagnetic ironoxide particles in combination with magnetic cell separation technique (MACS). Cells were analyzed with fluorescent activated cell sorting techniques (FACS) for the expression pattern of PSA-NCAM, compared with the expression of the surface carbohydrate epitope A2B5 and an antibody that recognizes the iron-containing particle in the freshly isolated neural progenitor cells. Additionally, the cells were cultured in a serum free medium supplemented with epidermal growth factor and basic fibroblast growth factor. For high resolution magnetic resonance imaging (MRI) we compared the signal intensities of cells with magnetically labeled surface-receptors to cells that are extracellularly labeled and additionally transfected with iron-containing particles *in vitro*. The results indicate that magnetically labeling only the PSA-NCAM receptor is not sufficient for high-resolution imaging purposes. Therefore it is necessary to increase the cellular iron content with an independent method. In addition to the *in vitro* studies we performed cell transplantations into the cortex of three to 6-week-old CD1-mice and analyzed these animals with 11.7 Tesla and 7.0 Tesla (high field) MRI scanners. Here we present a method for the enrichment of neural precursor cells using the MACS-technique and a highly effective contrast enhancement method for *in vivo* magnetic resonance imaging studies.

88.

Autologous stem cells for spinal cord injury

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Keywords: spinal injury, stem cells

We studied 100 patients with spinal cord injury after autologous stem cell injection in the spinal fluid with a follow up of 6 months post-stem cell injection.

There were 69 males and 31 females, age ranging from 8 to 55 years. Time after spinal injury ranged from 11 years to 3 months (average: 4.5 years). The level of injury ranged from upper thoracic (T1–T7): 34 patients; lower thoracic: 45 patients; lumbar: 12 patients; cervical: 9 patients. All patients had an MRI scan, urodynamic study and somatosensory evoked potential tests before and 3 months after stem cell injection.

A total of 80% of patients had grade zero power in the lower limbs and therest had grade 1–2 power before stem cell injections.

We extracted CD34 and CD133 marked stem cells from 100 ml of bone marrow aspirate using Ficoll gradient method with cell counting done using flowcytometry. In aseptic conditions, 15 ml of the stem cell concentrate was injected into the lumbar spinal fluid. The CD34/CD45 counts ranged from 120 to 500 million cells in the total volume.

After 6 months, post-injection, eight patients had more than two grades of motor power improvement, 12 had sensory tactile and pain perception improvement and eight had objective improvement in bladder control and bladder muscle contractility. A total of 12 patients had reported or observed improvement in neurological status. Of patients, 85% who had motor improvement had lesions below T8. MRI, somatosensory-evoked potential and urodynamic study data will be presented.

Conclusion

This study shows that quantative and qualitative improvement in the neurological status of paralyzed patients after spinal cord injury is possible after autologous hematological stem cell injections in select patients. Further studies to identify mechanism of action of stem cells on nerve tissue both *in vivo* and *in vitro* will be necessary to confirm the above results.

89.

The chemokine stromal derived factor-1 (SDF-1) induces 'homing' of human umbilical cord blood cells to a hypoxic-ischemic lesion in the rat brain

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Keywords: chemotactic factor, hypoxic-ischemic brain damage, SDF-1, umbilical cord blood

Previous studies have shown that transplanted human umbilical cord blood (hUCB)-derived mononuclear cells exert beneficial effects in various animal models of CNS impairments. The presence of hUCB cells at the lesion site seems to be a major prerequisite for their therapeutic effect; however, the mechanisms of cell 'homing' are still unclear. In this study, we focussed on elucidating mechanisms underlying the specific migration of hUCB-derived mononuclear cells to a hypoxic–ischemic lesion in the perinatal rat brain.

The presence of chemotactic signals at the lesion site is one possibility to induce cell 'homing'. The CXC chemokine stromal derived factor (SDF)-1 is a putative candidate of chemotactic factors, and was previously shown to be a potent chemoattractant for other stem and progenitor cells.

We examined the spatial and temporal expression of SDF-1 in brain hemispheres with or without hypoxic–ischemic lesion at two different time points. SDF-1 expression was substantially increased at the lesion site during the investigated period of 14 days after the insult. Furthermore, we were able to detect HLA-positive hUCB cells mainly in SDF-1-expressing brain regions and demonstrated that these cells express the SDF-1 receptor CXCR4, on their surface. The functional implication of SDF-1 in directing hUCB cell migration was determined by application of neutralizing SDF-1 antibodies *in vivo*, resulting in a reduced number of hUCB-derived mononuclear cells residing at the lesion site.

With these functional effects, together with the observed timing and location of its expression, the involvement of the chemokine SDF-1 in hUCB cell 'homing' seems conceivable.

90.

Transmigration and invasion of mesenchymal stem cells

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Keywords: invasion, mesenchymal stem cells, transmigration

Human adult bone marrow-derived mesenchymal stem cells (MSCs) are in the focus of scientific interest because they differentiate into various mesenchymal tissues. MSCs are not only used in the field of tissue engineering but also as a potential therapy for the regeneration of infarcted myocardial tissue. For a successful therapy, MSCs must transmigrate across the endothelium to exit the blood circulation and finally invade into their target tissue. To date, most of the underlying mechanisms of transmigration and invasion remain unelucidated. Improving our knowledge on these core processes might elevate the efficiency of stem cell therapy. Hence, our aim was to characterize key mechanisms involved in transmigration and invasion of MSCs.

Co-cultivation experiments with endothelial monolayer and MSCs infer that MSCs integrate into the endothelial monolayer. However, the time course of integration depends on the endothelial phenotype. Thus, a variable capacity for transmigration exists within the vasculature. Additionally, three-dimensional systems and *in vivo* experiments revealed that MSCs penetrate the endothelium and invade into the surrounding tissue via plasmic podia. Furthermore, to identify cell adherence molecules involved in the interaction, blockade experiments with anti-VLA-4 (integrin $\alpha 4/\beta 1$) and anti-VCAM-1 (CD106) were carried out and resulted in a significant slowdown of integration of MSCs into the endothelial monolayer. Thus, we assume that the VLA-4/VCAM-1 interaction plays a key role in the transmigration of stem cells across the endothelial barrier. In addition, *in situ* zymography of cardiac tissue infer activation of gelatinases at sites of MSC invasion.

In conclusion, the endothelial phenotype, formation of plasmic podia, VCAM-1/VLA-4 and gelatinase secretion are functional key players involved in transmigration and invasion of MSCs.

ELSI

92.

Avoiding totipotency in human organisms as a solution to ethical objections concerning the generation of pluripotent stem cells: feasible, necessary, ready for social consensus?

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Keywords: ANT, iPS, somatic cell nuclear transfer, totipotency, ethics

Ethical concerns have accompanyed stem cell research ever since human cells became involved. The most severe and fundamental questions arise from the destruction of early human embryos for the generation of pluripotent embryonic stem cells leading to prohibition of this procedure in many countries. Within the scope of the Stem Cell Act (2002), German legislation increasingly focused on the criterion of totipotency by protecting 'any human totipotent cell which has the potential to divide and to develop into a human being if the necessary conditions prevail'. Thereby, a descriptive terminus derived from classical developmental biology has finally turned normative.

Beside the central question of whether totipotency was a reasonable criterion for protecting human organisms, many experimental tricks have been proposed to bypass totipotent stages in development. These modifications of somatic cell nuclear transfer were thought to allow public funding in the US or to elude restrictive legislation. Although human organisms arising from somatic cell nuclear transfer are not proven to be totipotent, altered nuclear transfer was suggested and proof of principle shown to be successful. Alternatively, procuring embryonic stem cells from single blastomeres without destroying an embryo was demonstrated to be feasible. However, doubts arise as the cells used might still be totipotent. Similarly questionable is the use of probably organismic dead embryos. Ethical reasoning revealed all of these proposals not convincing as the potential of used organisms cannot be predicted securely. Furthermore, other embryo-like entities like hybrids which arise from interspecies somatic cell nuclear transfer, chimeras and manipulated parthenotes bear even greater philosophical and ethical problems to be solved.

A real solution for ethical problems relating to the generation of pluripotent cells could be to directly reprogram somatic cells. Although this idea has been for a long time, technical difficulties are foiling this approach so far. Successful direct reprogramming of murine fibroblasts might be a first step towards overcoming these difficulties. Induced pluripotent stem cells will most probably be suitable for ethical and social consensus because the creation and destruction of totipotent developmental stages can be avoided. But even if direct reprogramming in human tissue should be achieved in conceivable time, the establishment of this technique will not be possible without further use of human embryonic organisms. Thus, we still have to face the same question: is totipotency an adequate criterion for legislation?

Empirical data clearly shows that social consensus in Germany tends to a more liberal regulation similar to the one established in the UK. To arrive at a reasonable decision we have to consider particularly how to treat human embryos in general. There are absolutely no protective measures for pre-implantation embryos *in vivo* as is clearly shown by the widely accepted methods of inhibiting nidation. Therefore, a diverging valuation *in vitro* has to be justified in a comprehensible way.

93.

Stem cells in clinical neuroscience: is there a common ethical position?

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Keywords: neuroethics, neurology, neurosciences, psychiatry

Introduction

Since the first isolation of embryonic stem cells (SC) from the inner cell mass of human blastocysts [1], a broad range of methods for the generation of pluripotent and multipotent SC has been established. Most widely used sources for SC are embryos from *in vitro* fertilization, aborted fetuses, umbilical cord blood and adult stem cells (e.g., from bone marrow). Additional sources include somatic nuclear transfer, reprogramming of adult somatic cells, and the post-mortem isolation of stem cells from adult brain tissue. Research has progressed from basic science to the conduction of clinical trials on the application of SC in neurological diseases like amyotrophic lateral sclerosis or multiple sclerosis. Several conflicting ethical positions concerning generation and application of SC have been developed, leading to incompatible and sometimes internally inconsequent recommendations depending on the country where studies are conducted.

The goal of the current paper is to summarize ethical problems with the generation and application of SC in the clinical neurosciences and to identify possible common standards.

Methods

A concise review of the current discussion in the biomedical and ethical field was performed.

Results

Candidate neurological diseases for SC therapy are Parkinson's disease, Huntington's chorea, cerebral infarction, amyotrophic lateral sclerosis, multiple sclerosis and spinal cord injury. Two recent position papers from the American Academy of Neurology and the American Neurological Association [2] as well as the World Federation of Neurology [3] demonstrate that the theoretical discussion has reached clinical neurology. Although there are also psychiatric diseases like Alzheimer's disease that are potential candidates for SC treatment, no consensus statements are available from psychiatric associations.

Common ethical problems arise from the source of SC (consumption of embryos and usage of aborted fetuses), from stem cell properties (totipotency, theoretically making possible the generation, manipulation and – in the case of somatic nuclear transfer and reprogramming of adult somatic cells – duplication of human organisms), and from adverse events resulting from autologous cell transplantation (e.g., induction of tumors). Ethical questions discussed include the moral status of the human blastocyst, contamination of a person's individuality by transplantation of neuronal cells or generation of genomically identical totipotent cells, and the induction of inadequate expectations about the therapeutic applicability of SC by improper reporting of results from scientific research.

Conclusion

There is already a common basis concerning the use of multipotent SC obtained, for example, from umbilical cord blood or bone marrow, allowing the conduction of clinical trials. Concerning generation and application of pluripotent SCs, a minimal standard is developing in the Anglo-American countries. Awareness for SC issues in psychiatry has to catch up as clinical trials, for example, in Alzheimer's disease can be anticipated. This discussion should be integrated in the emerging field of neuroethics.

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