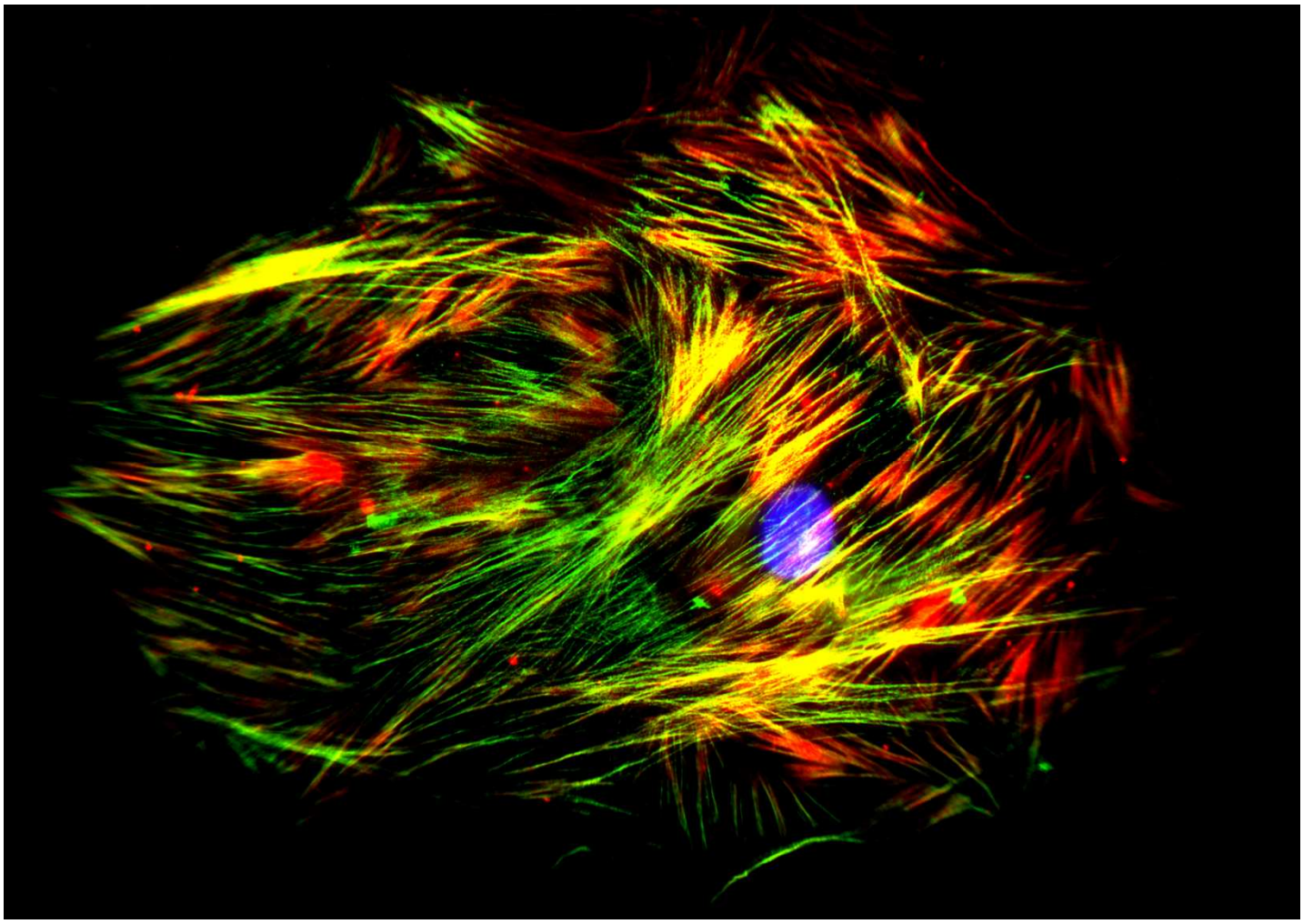
DAG VAN DE BIOMEDICI

2011



Vrije Universiteit Brussel

Geneeskunde & Farmacie

GF

Beste studenten en participanten,

De onderwijscommissie Biomedische Wetenschappen wil u graag welkom heten op de eerste 'Dag van de Biomedici'.

Met dit initiatief willen wij alle studenten Biomedische Wetenschappen de kans geven om in contact te komen met het onderzoek dat verricht wordt op de VUB campus Jette. Er komt een breed spectrum van onderwerpen aan bod, waaronder diabetes, epilepsie, multiple myeloma en nog veel meer. Deze dag is een uitgelezen moment voor de jongere generatie om al eens na te denken over hun toekomstige stages.

Deze dag staat enerzijds in het teken van het biomedisch onderzoek dat door onze 2de master studenten verricht wordt ter voorbereiding van hun thesis. Naast een voormiddag gevuld met presentaties door onze 2de master studenten, mag u in de namiddag sprekers verwachten uit een brede waaier van beroepen. Wij hebben het plezier om u naast een VUB-alumnus van onze richting (Dr. Ingeborg Heirman – Merck) werkzaam in de industrie, ook prof. Dirk Van Varenbergh voor te stellen die een woordje uitleg komt geven over de forensische wetenschapsmogelijkheden. Bovendien hebben we dankzij de firma SGS Life Science Services de mogelijkheid om wat meer te weten te komen over de rol van een Clinical Research Associate (CRA) en een Clinical Data Reviewer (CDR) met betrekking tot klinische studies.

Tot slot willen wij u uitnodigen om deel te nemen aan de labdrink, waar wij u als jonge student de mogelijkheid bieden om een praatje te slaan met de master studenten en waarbij de master studenten de kans krijgen om verdere informatie in te winnen bij het beroepenveld.

Happy Meeting

Programma: Deel I

08.45 - 09.15	**Registratie**
09.15 - 09.30	**Welkomstwoord** door prof. Karin Vanderkerken
09.30 - 09.45	**Sarah Akbib:** Fenotypering van beta-cellen door analyse van hun translationeel actief mRNA
09.45 - 10.00	**Adil El Taghdouini:** Selectieve aflevering van antifibrotische middelen aan leverstellaatcellen door middel van liposomen
10.00 - 10.15	**Jessica Coppens:** Onderzoek naar de rol van neuropeptiden in limbische epilepsie
10.15 - 10.30	**Elina Dakaeva:** Increased replication in human donor pancreas is associated with increased leucocytic infiltration and increased expression of inflammation related genes
10.30 - 10.45	**Kim De Veirman:** Targeting osteoblastogenesis of mesenchymal stem cells by novel multiple myeloma drugs
10.45 - 11.00	**Gabrielle Dethioux:** Milde hypothermiebehandeling na transiënte focale cerebrale ischemie in ratten
11.00 - 11.15	**Frisdrank - Pauze**
11.15 - 11.30	**Tugba Bilgec:** Studie van de epigenetische aspecten na spermatogoniale stamceltransplantatie en testiculaire weefseltransplantatie in een muismodel
11.30 - 11.45	**Carlien Geldof:** Epigenetische analyse van specifieke genen in kinderen geboren na geassisteerde voorplanting en na spontane conceptie
11.45 - 12.00	**Bart Legein:** Het effect van Tamoxifen op de beta-celproliferatie in verschillende modellen
12.00 - 12.15	**Susanne Lub:** Ontrafeling van bortezomib resistentie mechanisme in multiple myeloma
12.15 - 12.30	**Stijn Van Langenhoven:** Stapsgewijze differentiatie van muis embryonale stamcellen tot multipotente pancreatische progenitoren

Programma: Deel II

12.30 - 14.00	**Lunch**
14.00 - 14.15	**Susanne Lub (2de master BMW):** Een stapje buiten de VUB!
14.15 - 14.30	**Dhr. Paul Ysebaert - SGS Life Science Services:** De rol van een Clinical Research Associate (CRA)
14.30 - 14.45	**Mevr. Els Van Haver - SGS Life Science Services:** De rol van een Clinical Data Reviewer (CDR)
14.45 – 15.00	**Dr. Lies Peeters - Ambtenaar bij de Hoge Gezondheidsraad (HGR):** Werken voor de Hoge Gezondheidsraad, wat houdt het in?
15.00 - 15.15	**Frisdrank - Pauze**
15.15 - 15.30	**Dr. Ingeborg Heirman (BMW Alumnus VUB) - Merck:** De meerwaarde van een doctoraat voor het management van vroege fase I klinische studies
15.30 - 15.45	**Drs. Lien Thoen (BMW Alumnus VUB) - PhD student VUB:** Hoe een IWT aanvragen ?
15.45 – 16.00	**Prof. Dirk Van Varenbergh - Anatomo-Pathologie UZ Brussel - VUB:** Raakvlakken van de forensische geneeskunde met andere disciplines in het forensisch onderzoek
16.00 - 18.00	**Labdrink**

Abstracts 2de Master BMW voor het Maastricht Medical Students Research Conference 2011 (MMSRC)

Sinds 2003 nemen de studenten tweede master Biomedische Wetenschappen deel aan het Maastricht Medical Students Research Conference. Op dit internationale congres krijgen (bio)medische studenten de mogelijkheid om hun eigen wetenschappelijk onderzoek voor te stellen. De auteurs met de beste ingezonden abstracts krijgen de mogelijkheid tot een orale presentatie en de andere auteurs stellen hun onderzoek voor met een poster presentatie. Zowel de orale als de poster presentatie wordt beoordeeld door een internationale jury.

Verschillende van onze studenten behaalden reeds prijzen op het MMSRC:

2010 - **Lien Thoen** behaalde de prijs van beste orale presentatie met 'The role of lipolysis in hepatic stellate cell activation'. **Liesbeth Bieghs** kaapte de publieksprijs voor de beste poster presentatie weg met 'Forodesine reduces proliferation and induces apoptosis in multiple myeloma'.

2009 - **Violette Coppens** behaalde de prijs van de beste orale presentatie met 'Human endothelial cells improve the outcome of the experimental islet transplantation'. **Sander Van Lint** kaapte de publieksprijs voor de beste poster presentatie weg met 'Development of a multiplex PCR for occurence of uniparental disomy in all chromosomes of human embryonic stem cell lines'.

2007 - **Inge Neels** kreeg de prijs voor de beste poster presentatie en **Miguel Lemaire** ontving de publieksprijs voor de beste poster.

2005 - **Christelle Orlando** kreeg de prijs voor de beste mondelinge presentatie en **Mieke Geens** ontving de publieksprijs voor beste poster. **Tessa Dieltjens** nam in deze categorie de tweede plaats voor haar rekening.

2003 - **An Verloes** behaalde met de mondelinge presentatie van haar eindwerk de eerste prijs. **Pieter Goossens** behaalde de eerste prijs voor de beste poster presentatie.

Phenotyping of Pancreatic Beta Cells through Analysis of their Translational Active mRNA

Authors: *Sara Akbib*, Zhidong Ling

Department of Metabolism and Endocrinology, Diabetes Research Center, Vrije Universiteit Brussel, Belgium

Introduction: Normal pancreatic beta cells rapidly secrete insulin in response to an acute increase in blood glucose levels. Beta cells are able to adapt their functions to increased insulin demand by changing phenotype and/or by increasing number. Translation processes control beta cell phenotype. The aim of present study is to develop a technique to isolate translationally active mRNA. Actively translating mRNAs are associated with polysomes and newly synthesized polypeptides, which in turn are associated with chaperone molecules like HSP70s. Such chaperones can provide an anchor to separate polysome-associated mRNAs from free mRNAs. Recently, Kudo et al showed the possibility to isolate HSP70-associated translationally active mRNA using antibody affinity capture beads. In the present study, we want to apply this new technique to insulin-producing cells. The first step is to optimize immunoprecipitation (IP) conditions. The IP condition with the highest efficacy and reproducibility will be used for mRNA isolation.

Material and Methods: INS-1 cells were used in the study. We first examined HSP70 expression by Western blot using five different anti-HSP70 antibodies at different concentrations: mouse and rabbit HSP70 (Abcam), mouse and rabbit HSP70 (Millipore) and rabbit HSP70 (Cell Signaling). We then tested three different Dynabeads (M280 sheep anti-mouse IgG, co-IP kit and protein G kit) for IP using selected antibodies. Different ratios of beads/antibodies/cells were tested. After IP, the abundance of immunoprecipitable HSP70 was examined by Western blot and compared to the abundance of same protein using samel number of cells but without IP.

Results: In INS-1 cells, the highest abundances of HSP70 were detected by Western blot using mHSP70 (Abcam) and RbHSP70 (Millipore). The other Abs showed no detection or low specificity. Dynabeads from co-IP kit and protein G kit gave a poor result. Dynabeads M280 coupled with mHSP70 gave a positive result for WB after IP. However, the immunoprecipitable HSP70 is only <20 % of their initial content (without IP).

Discussion and Conclusion: HSP70 in INS-1 cells can be captured by Dynabeads M280 coupled with mHSP70 (Abcam). However, IP efficacy is low. In order to ensue the efficient capture of HSP70 bound mRNA, it is necessary to further optimize IP conditions.

Study of Epigenetic Modifications in Germ Cells after Spermatogonial Stem Cell Transplantation

Authors: *Tuğba Bilgec*, Dorien Van Saen, Herman Tournaye, Ellen Goossens

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Introduction: Fertility preservation is one of the key issues for young cancer patients undergoing gonadotoxic treatments. The current method of preserving fertility for adult men, sperm cryopreservation, is not possible for prepubertal boys due to the lack of sperm cells. For these boys, spermatogonial stem cell transplantation was suggested as a fertility preservation technique. This method involves the autologous intra-testicular transplantation of a cryopreserved testicular cell suspension after therapy. A clinical application requires an efficient and safe transplantation protocol. Since it has been demonstrated that an altered epigenetic pattern causes impaired spermatogenesis, the possible effect of transplantation on epigenetic characteristics was evaluated. The overall methylation pattern and the expression of DNA methyltransferases 1 and 3a were examined in a cell-type stage-specific manner.

Material and Methods: Donor stem cells were obtained from prepubertal mice and transplanted in 13 infertile, adult W/W receptor mice. At least four months after transplantation, testes were analyzed by immunohistochemistry and data were compared with prepubertal and adult fertile mice.

Results: The overall methylation pattern after spermatogonial stem cell transplantation was similar to that of the control group. Results revealed that spermatogonia from stage V - VIII and spermatogonia and elongating spermatids from stage IX - XII stained positive. Comparison of the stage specific expression of DNA methyltransferase 1, which was expressed in round spermatids from stage I – IV, in primary spermatocytes (stage VII - VIII) and in round spermatids from stage IX - XII showed that there is no difference between both groups. DNA methyltransferase 3a was only expressed in spermatogonia from stage V - IX in both groups.

Discussion and Conclusions: Although further analyses are needed to make final conclusions, this qualitative study shows that there is no important change in DNA methylation after spermatogonial stem cell transplantation. In the future, quantitative studies have to be realized to confirm these results.

The Role of Ghrelin in Limbic Epilepsy

Authors: *Jessica Coppens*, Jeanelle Portelli, Ellen Loyens, Ilse Smolders

Department of Pharmaceutical Chemistry & Drug Analysis (FASC), Center for Neuroscience, Vrije Universiteit Brussel, Belgium

Introduction: Since approximately 30% of epilepsy patients are resistant to the present anti-epileptic drugs, it is of great importance to find novel drug targets. Ghrelin is a 28-amino acid peptide, known as the endogenous ligand of the growth hormone secretagogue receptor 1a (GHSR1a). A handful of studies reveal that ghrelin has anticonvulsant properties in rodents and that plasma ghrelin levels are altered in epilepsy patients. Our aim was to clarify the mechanism through which ghrelin exerts its anticonvulsant effect.

Material and Methods: Male albino Wistar rats underwent a 260 min intrahippocampal microperfusion of ghrelin (0.01-10 microM), truncated ghrelin (0.1-100 microM) or the GHSR1a antagonist A778193 (10-50 microM) via a stereotactically implanted microdialysis probe. At the 120th min of compound microperfusion, 12 mM pilocarpine was co-administered for 40 min to induce seizures. Seizure-related behavioural changes were scored. Furthermore, male GHSR1a-/- (KO) and GHSR1a+/+ (WT) mice were compared for genotype differences in seizure thresholds. They received an intraperitoneal injection of saline 30 min prior intravenous pilocarpine tail infusion (24 mg/ml). A separate KO mice group was injected ghrelin (1.8 microg/g) instead of saline. Seizure thresholds were determined for the stages of intravenous pilocarpine-induced behavioral changes.

Results: Ghrelin dose-dependently inhibited pilocarpine-induced seizures in rats with a lowest effective dose of 0.1 microM ghrelin. Unexpectedly, A778193 was also able to inhibit pilocarpine-induced seizures. Different doses of truncated ghrelin, which is 1000 fold less able to desensitize GHSR1a than ghrelin, show that truncated ghrelin (0.1-10 microM) was not able to protect the rats against pilocarpine-induced seizures. To explicitly check whether GHSR1a is involved in epilepsy, we compared pilocarpine-induced seizure thresholds of KO and WT mice. Seizure thresholds in KO mice were higher compared to their WT littermates, which is in line with the results of A778193. No differences were observed between KO mice treated with ghrelin and KO mice treated with saline.

Discussion and Conclusion: Our results show that the GHSR1a is implicated in epilepsy. Blockage of the receptor is necessary to obtain an anticonvulsant effect and we suggest that ghrelin works on the GHSR1a through desensitization.

Increased Replication in Human Donor Pancreas is Associated with Increased Leucocytic Infiltration and Increased Expression of the Stem Cell Marker Olfactomedin 4 (OLFM4) and the Transcription Factor Apolipoprotein C1 (APOC1)

Authors: *Elina Dakaeva*, Peter In't Veld

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Introduction: Type 1 diabetes mellitus is characterized by chronic hyperglycemia and an absolute insulin deficiency, caused by autoimmune destruction of the pancreatic beta-cells. Regenerating the endogenous beta-cell mass might form an important new therapy. We recently found that adult human beta-cells retain a substantial replicative potential and that Ki67+ beta-cells are induced under conditions associated with prolonged life support. We are investigating the hypothesis that (local) inflammation leads to a strong increase of beta-cell replication.

Material and Methods: We compared twenty patients with a high level of beta-cell replication (test group) to twenty matched controls with low replication (controls). All patients in the test group were <25 yrs and were mechanically respirated for >3 days. The inflammatory infiltration was investigated by morphometry and immunophenotyping on pancreatic sections. Replication was quantified using immunohistochemistry for Ki67 and cell type specific markers. Cryo-preserved human donor pancreas was used to isolate mRNA and study gene expression patterns with Affymetrix U133 arrays.

Results: The test group showed a 7-30 fold increase of replication in alpha, ductal, endothelial and acinar cells. This was accompanied by a significantly higher level of pancreatic infiltration by CD3+, CD20+, CD45+ leucocytes and CD68+ monocytes/macrophages, localized around the blood vessels/interlobular septa and spread throughout the pancreas, respectively. The monocytes/macrophages possessed a M2 phenotype. Analysis of gene expression in mRNA isolated from intact pancreas of test group, revealed 188 upregulated genes, predominantly from inflammation and growth related pathways with overexpression of OLFM4 and APOC1 genes being most prominent.

Discussion and Conclusion: Increased beta-cell replication was found to be associated with an increased replication of all pancreatic cell types and with an increased level of inflammatory infiltration, including an increased expression of M2 monocytes/macrophages. These results are supported by gene expression analysis showing upregulation of inflammation and growth related genes. It is hypothesized that conditions of prolonged life support lead to hypoxia-induced cell damage, inflammatory infiltration and local release of factors with a repair/growth promoting effect.

Targeting Osteoblastogenesis of Mesenchymal Stem Cells (MSC) by Novel Multiple Myeloma Drugs (HDAC inhibitor- Vorinostat/ Proteasome inhibitor- Bortezomib)

Authors: *Kim De Veirman*[1], Song Xu[1], Gaia Cecilia Santini[1], Karin Vanderkerken[2], Ivan Van Riet[1]

[1]Stem Cell Laboratory-Division Clinical Hematology, Universitair Ziekenhuis Brussel, Belgium

[2]Department of Hematology and Immunology, Vrije Universiteit Brussel, Belgium

Introduction: MSC are able to differentiate into osteoblasts, adipocytes and chondrocytes, and gained wide popularity in regenerative medicine. Myeloma (MM) is a plasma cell cancer and up to 90% of the patients develop bone lesions caused by an imbalance between osteoblast and osteoclast activity. Recent reports revealed an important role of histone deacetylase inhibitors (HDACi) in bone turnover by stimulation of osteoblast formation and maturation in vitro. The purpose of this study is to examine the potential of suberoylanilide hydroxamic acid (SAHA), the only HDACi currently used in clinical phase I/II trails for MM patients, to induce osteoblastogenesis in vitro and in vivo. Moreover, the combination with Bortezomib (Bzb), a drug for which it has been proven that it promotes MSC osteogenesis, will be tested.

Material and Methods: MSC were obtained from bone marrow samples of MM patients and control subjects. The optimal concentration of SAHA was determined using viability assays on human MM cell lines (e.g. RPMI8226) and MSC. Epigenetic modulations were detected by western blot for HDAC1/4, p21 and acetyl-histone 3 (ac-H3). The effect of SAHA on osteoblasts was investigated by alkaline phosphatase (ALP) staining, Alizarin Red S staining and PCR.

Results: We found SAHA at a concentration of 1 micromolar had no significant effect on the viability of MSC (IC50 15.57 micromolar), though the concentration was high enough to kill MM cells. In vitro studies showed SAHA 1 micromolar increased, in MSC from both MM patients and normal donors, the activity of ALP, which is an early marker of osteoblast differentiation. This osteogenesis-promoting effect was confirmed by PCR analysis for osteogenic markers and matrix mineralization. Importantly, we observed SAHA upregulates Runx2 expression, a key transcription factor of osteoblast formation. SAHA also increased ac-H3 and p21 expression of MSC, but suppressed HDAC1 and HDAC4 activity, mimicking natural epigenetic alteration during MSC osteogenic differentiation. Synergistic effects on osteoblastogenesis were found by combining SAHA 100nM and Bzb 2nM.

Discussion and Conclusions: In conclusion, our data indicate that SAHA alone or combined with Bzb stimulates osteoblast formation in vitro. Further studies in naive and 5T33MM mice will be proceeded to confirm the osteogenesis-promoting potential also in vivo.

Effect of Mild Hypothermia after Cerebral Ischemia in a Rat-model

Authors: *Gabrielle Dethioux*, Tine Zgavc, Yvette Michotte, Sophie Sarre, Said Hachimi-Idrissi

Critical Care Department and Cerebral Resuscitation Research Group - Department of Pharmaceutical Chemistry and Drug Analysis, Center for Neuroscience, Vrije Universiteit Brussel, Belgium

Introduction: Stroke is the third leading cause of death and permanent disability worldwide. The only FDA-approved therapy is the thrombolytic approach using recombinant tissue type plasminogen activator (rt-PA). Unfortunately, the time window is narrow. Hypothermia has shown to confer a significant degree of neuroprotection after cerebral ischemia. It interacts with several pathways of stroke (inflammation, apoptosis, excitotoxicity, etc). The aim of the study is to evaluate the neuroprotective effect of a hypothermic treatment in a rat-model of stroke. The severity of the ischemic insult and the possible correlation between the infarct size, the neurological deficit score (NDS) and the concentration biomarkers (such as S100-beta, glial fibrillary acidic protein (GFAP) and metalloproteinase-9 (MMP-9)) in blood will be investigated.

Material and Methods: Two probes, one for Et-1 and one for the temperature, are implanted via stereotactic surgery into the brain of Wistar rats. The cerebral ischemic insult is induced by injecting Et-1, a potent vasoconstrictor, near the middel cerebral artery. Mild hypothermia (33°C) is induced 20 minutes after Et-1 injection during 2 hours. The results are compared with the normothermic (37°C) and sham (37°C) groups. The infarct size and the behavioural outcome are assessed 24 hours after induction of the insult using respectively cresyl-violet staining and the NDS. Blood samples are taken at several timepoints; before and after Et-1 injection, after treatment, at sacrifice. Bloodmarkers (MMP-9, GFAP and S100-beta) are analyzed using ELISA-kits.

Results: The NDS improved in the hypothermic group compared to the normothermic group. Hypothermia significantly reduced the infarct size in comparison with the normothermic group. Analysis of the bloodsamples with ELISA are ongoing.

Discussion and Conclusion: Post-ischemic induction of mild hypothermia is a promising neuroprotective therapy, it reduces the infarct size and improves the neurological deficit. The evaluation of bloodmarkers could be useful in order to know if the patient would still benefit a hypothermic treatment and to assess the clinical outcome.

Selective Delivery of Doxorubicin to Hepatic Stellate Cells by Using Vitamin A-Coupled Liposomes

Authors: *Adil El Taghdouini*, Liesbeth Peeters, Inge Mannaerts, Leo A. van Grunsven

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Introduction: The conversion of a quiescent vitamin A-rich hepatic stellate cell (HSC) to one that is contractile, fibrogenic and proliferating is a key event during fibrogenesis. Therefore, pharmacological intervention with an antiproliferative drug may result in antifibrotic effects. Doxorubicin (DOX), an anthracyclin antibiotic, is a potent inhibitor of cell proliferation and is widely used as chemotherapeutic. Recently, it has been reported that DOX can decrease liver fibrosis in the bile duct ligation rat model. Serious adverse effects, i.e. cardiotoxicity associated with the use of DOX precludes its clinical use for the treatment of liver fibrosis. In the present study, we evaluated the anti-fibrotic effect of DOX by selectively targeting it to HSCs using vitamin A-coupled liposomes (LipoDox-VA).

Material and Methods: BALB/c mice were subjected to CCl4 injections to induce liver fibrosis. After fibrosis was induced, the mice received 3 injections of free DOX or LipoDox-VA, in a dose of 0,7 mg/kg, and were euthanized 24h after the last DOX injection. Total RNA from liver and heart tissue was extracted, reverse-transcribed and subjected to quantitative PCR (qPCR) to determine the relative RNA levels in liver and heart for genes characteristic of HSC activation (i.e. alpha smooth muscle actin (aSMA) and collagen type 1 (procol1a1)) and markers of cardiotoxicity (i.e. atrial natriuretic peptide (ANP) and manganese superoxide dismutase (MnSOD)), respectively. Liver tissue samples were analyzed for matrix deposition and HSC markers by immunohistochemistry. Collagen positive areas in the liver were quantified after picrosirius red staining using NIH image J software.

Results: After treatment with LipoDox-VA, the animals showed less fibrosis evidenced by a reduced collagen stained area in the liver and decreased RNA levels of aSMA and desmin, a marker of HSCs. After treatment with free DOX, the collagen stained area was not reduced. There was no significant difference in ANP and MnSOD expression in heart tissue of mice treated with free DOX or LipoDox-VA.

Discussion and Conclusions: We show that selective delivery of DOX to HSCs is possible by using vitamin A-coupled liposomes, resulting in a reversal of fibrosis in the CCl4-induced liver injury model. The DOX dose used does not allow us to discriminate between free DOX and LipoDox-VA induced cardiotoxicity.

Epigenetic Analysis of Specific Genes in Children Born after Assisted Reproductive Technologies or Conceived Spontaneously

Authors: *Carlien Geldof* [1], Carrie Williams [2], Alastair Sutcliffe [2], Maryse Bonduelle [3], Martine De Rycke [3]

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Introduction: Assisted reproductive technologies (ART) provide a great benefit for many couples struggling with infertility problems. Last few years, an association between ART and epigenetic disorders has been suspected. Human and animal studies suggest epigenetic deregulation during early development, which may lead to a change of the phenotype such as low birth weight or an increased prevalence of metabolic and cardiovascular disease later in life. The epigenetic analysis of blood samples from children born after ART or spontaneous conception will give information about the influence of ART on epigenetics. We will investigate the expression and DNA methylation patterns of specific genes important for fetal growth and nutrition.

Material and Methods: Blood samples from children conceived after ART (n=52) and from spontaneously conceived children (n=28) were collected after informed consent in PAXGene Blood RNA or DNA Tubes. The children were part of an existing cohort from the UK, followed since birth. Blood samples were stored at -20°C and transferred frozen to Belgium. RNA was extracted for reverse transcription and subsequent Real-Time PCR analysis using specific gene expression assays for Hypoxanthine Phosphoribosyltransferase 1 (HPRT1, endogenous control), DNA methyltransferase 1 (DNMT1), Glucocorticoid receptor (NR3C1) and Peroxisome Proliferator Activated Receptor Alpha (PPARA). DNA was extracted for analysis of DNA methylation patterns for the same genes using bisulphite conversion and pyrosequencing. Pyrosequencing assays are performed by Varionostics.

Results: Gene expression levels were normalised against the endogenous control HPRT and relatively quantified using the ddCt method. The mean blood mRNA levels of DNMT1, NR3C1 and PPARA in the ART children showed 0.98, 0.97 and 1.27 fold change respectively compared to those of the children from spontaneous conception ($p=0.911$, $p=0.829$ and $p=0.109$ respectively). The results of DNA methylation patterns are still under investigation.

Discussion and Conclusion: No difference was observed in mRNA expression levels of DNMT1, NR3C1 and PPARA between children conceived after ART and children spontaneously conceived. The preliminary conclusion is that ART procedures do not seem to affect the epigenetic status of these genes.

Tamoxifen Inhibits Beta Cell Proliferation and Biases Beta Cell Neogenesis Mechanisms in Mouse Pancreas

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Introduction: Cell therapy is a promising approach to cure diabetes. However, severe shortage of transplantable donor beta cells hampers broad application and encourages intensive research for alternative sources of beta cells. Beta cell progenitor/stem cells (Ngn3+ cells) have recently been identified in mouse pancreas, but it is unclear whether these progenitors can generate new beta cells in vivo. In pancreas injury models, formation of new beta cells is studied by lineage tracing of (Ngn3+) endocrine progenitor cells and/or pre-existing beta cells. The CreERT system is widely used for genetic labeling of these specific pancreas cell subtypes, but also for cell ontogeny studies in other tissues. We examined whether tamoxifen (TAM), the ligand that is administered for inducing the nuclear translocation of Cre and thus for genetic labeling of the cells, by itself can affect neogenesis and proliferation.

Material and Methods: The effect of TAM on beta cell formation was studied in the pancreas of neonatal and adult mice. To stimulate beta cell neogenesis in adult mouse pancreas the organ was injured by partial duct ligation (PDL) or partial pancreatectomy (50% PPX). Beta cell proliferation was studied in transgenic (CreERT+) mice and in non-transgenic Balb/C mice by IHC for incorporated DNA analogs or for proliferation marker expression. Control tissues showing active cell cycling were liver and duodenum. QRT-PCR was performed to study the effect of TAM on the expression of the endocrine progenitor-specific master gene Ngn3.

Results: TAM exhibited a strong inhibitory effect on both endocrine beta- and exocrine duct cell proliferation following PDL (8 vs. 28% and 65 vs. 91%). TAM also inhibited beta cell proliferation in the normal pancreas (9 vs. 27%) and following 50% PPX (11 vs. 34%). While proliferation of hepatocytes was also inhibited by TAM (11 vs. 34%), TAM did not affect the proliferation of duodenal crypt cells (81 vs 82%). In addition to its negative influence on beta cell proliferation, TAM dramatically decreased the Ngn3 expression in pancreas after PDL and to a lesser extent the constitutive Ngn3 expression in duodenum.

Conclusions: Our data indicate that TAM inhibits beta cell proliferation and activation of Ngn3 gene expression and thus biases studies using CreERT-based lineage tracing.

Unraveling of Bortezomib Resistance Mechanisms in Multiple Myeloma

Authors: *Susanne Lub*, Eline Menu, Elke De Bruyne, Karin Vanderkerken, Els Van Valckenborgh

Department of Hematology and Immunology, Myeloma Centre Brussels, Vrije Universiteit Brussel, Belgium

Introduction: Multiple myeloma (MM) is a plasma cell cancer associated with an accumulation of tumor cells in the bone marrow (BM). Despite improvements in treatment by the discovery of new agents like bortezomib (bz, proteasoom inhibitor), MM is still incurable and most of the patients will relapse. Different populations are described which are clonally related to tumor. Some studies describe that CD138- (B cells) cells are a minor population containing MM clonogenic and therapy-resistant cells, others show that only CD138+ (plasma cells) cells are able to proliferate and engraft. The aim of the current project is to identify Bz resistancy among different subpopulations, including CD138- and CD138+ MM cells.

Material and Methods: For in vivo experiments the 5T33MM mouse model was used. Human MM cell lines RPMI8226 and Opm-2 were used for in vitro experiments. Populations were sorted by fluorescent activated cell sorting (FACS) and analysed for Bz response. Viability was measured by the CellTiter-Glo® Luminescent Viability Assay and apoptosis was measured by Caspase3/7 Glo® assays. Soluble CD138 (sCD138) was determined in the supernatants by ELISA. The expression of different markers was assessed with flow cytometry, western blot and real-time PCR.

Results: 5T33MMvv cells, isolated from the BM of diseased mice, were sorted in idiotype+ (tumor marker) CD138- and CD138+ populations to investigate in vitro sensitivity to Bz by measuring viability and caspase3/7 activation. CD138- cells tend to be more resistant to Bz. Subsequently, we investigated the phenotype of 5T33MMvv cells after in vivo treatment with Bz. The percentage of CD138- 5T33MM cells was significantly increased when mice were treated with Bz compared to vehicle, this was confirmed in vitro with human MM cell lines. An increased concentration of sCD138 in het supernatants of human MM cell lines after Bz treatment suggests that proteasome inhibition causes CD138 shedding. Furthermore RT-PCR results indicate that CD138- 5T33MMvv cells express more ABCG2, an ABC transporter involved in resistance. When human MM cell lines were treated with Bz the percentage of ABCG2+ cells was significantly increased.

Conclusion: From these results we can conclude that CD138- MM cells are more therapy-resistant and that ABCG2 expression changes after Bz treatment. The role of ABCG2 in Bz resistance will be further investigated.

From Embryonic Stem Cells to Pancreatic Beta-Cells by Extracellular Factors

Authors: *Stijn Van Langenhoven*, Josué Mfopou, Luc Bouwens

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Introduction: Directed differentiation of embryonic stem cells (ESCs) beholds the potential to create an unlimited source of insulin producing beta-cells for diabetes cell therapy. With respect to this, an in vitro differentiation protocol was established by our group for the generation of endocrine pancreatic progenitors from human ESCs (hESCs). Ongoing research is focused on their further differentiation into mature endocrine beta-cells. As a drawback, these ESC should go through all previous stages making maximal refinement and efficiency of major importance. To circumvent this issue we would like to create a proliferative PDX1+ progenitor pool from mouse ESC (mESC), which would allow increased screening capacity of pro-endocrine factors in the last differentiation steps. In addition, we would like to develop a clinical-grade protocol by replacing several biological agents by chemicals wherever possible.

Material and Methods: Undifferentiated mESCs E14 were maintained on mitomycin C-inactivated CF1-mouse fibroblasts and manually passaged every 3-4 days. Cells were differentiated in 4-,6-,24- or 48 well-plates with our protocol. At each stage, relevant markers were determined using qRT-PCR and immunofluorescence. Numerical data were analyzed in Prism software.

Results: A critical step in differentiating cells towards the pancreatic lineage is definitive endoderm (DE) formation, characterized by increased FoxA2 and Sox17 expression. Upon optimization, we managed to induce at least 60% of the mESC towards the DE. In a next step, these cells were successfully differentiated towards PDX1/NKX6.1+ pancreatic progenitors with efficiencies ranging up to 60%. Similar results were obtained when replacing biological agents by chemicals.

Discussion and Conclusions: We state that the main in vivo molecular events needed for generating pancreatic cell lineages have been unraveled and could be implemented in vitro. Doing so, we managed to generate pancreatic progenitor equivalents from mESC with our protocol, which suggests pathways conservation across species. To date we have insufficient knowledge of the beta-cell maturation process in order to get functional insulin-producing cells in vitro but success in establishing a PDX1+ progenitor pool from mESC will be an asset for investigating this process.

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