Material and Methods: We have compared the secretomes from senescent versus young fibroblasts using secretome proteomics, combined with Western-blotting, ELISA, RT-PCR and zymographies. A fraction of NHEK growth culture medium was replaced by fibroblast (either young or senescent)-conditioned medium to investigate the secretome impact on neoplastic initiation of NHEKs.
Results: Four major groups of proteins were modulated in senescent vs young fibroblast secretomes. The expressions of extracellular matrix collagens, SPARC, and decorin were strongly affected in senescent fibroblasts secretome; expression and activation of numerous metalloproteinases were promoted, while the expression of their inhibitors was reduced; growth factors (among which HGF/SF) and cytokines were overexpressed in association with the loss of anti-angiogenic molecules. Senescent fibroblasts could then relevantly contribute to a tumour-promoting environment. Hence, the replacement of a fraction of primary NEHKs' growth culture medium by conditioned medium from normal primary senescent fibroblasts induced a strong promotion of the neoplastic initiation from primary NHEKs emerging from senescence in our culture model. It led to the acquisition of enhanced migratory and scattering capacities, and the development of small clones in soft Agar.
Conclusions: These results point to the microenvironment of normal aging fibroblasts as a factor promoting initial changes in normal human keratinocytes emerging from replicative senescence in vitro that result in cancerous phenotype.

867 Long-term GLI1 expression induces mammary gland tumour formation in nulliparous transgenic mice

J.H. Norum[1], M. Kasper[1], V. Jaks[1], R. Toftgård[1]. [1]*Karolinska Institute, Department of Biosciences and Nutrition, Stockholm, Sweden*

Background: The main effectors of the Hedgehog (Hh) signalling pathway are the zinc finger transcription factors of the GLI family. In human breast cancer, up regulation of GLI1 expression correlates with unfavourable overall survival. We have previously shown that multiparous conditional transgenic mice expressing GLI1 develop hyperplastic lesions and tumours. Furthermore, the Hh pathway is thought to be involved in the regulation and maintenance of CD44 positive breast cancer stem cells. Skin stem cells with active Hh signalling pathway as well as intestinal stem cells express the orphan G protein coupled receptor LGR5. The expression pattern and role of LGR5 in mammary gland tissue and cancer is not known.
Material and Methods: GLI1 expression was induced, up to 108 weeks, in female transgenic mice (MMTVrtTA;TREGLI1 and MMTVrtTA;TREGLI1;Lgr5-LacZ). The mice were monitored for the occurrence of tumours. Palpable tumours and hyperplastic lesions developed in the mice with induced GLI1 expression. Normal and tumour tissue were analysed by immunohistochemistry.
Results: Hyperplastic lesions and palpable mammary gland tumours, including solid and acinar adenocarcinomas, developed in the nulliparous mice after long-term low level GLI1 expression. Both cytokeratin 5 (K5) and cytokeratin 6 (K6) positive tumour cells were detected. Only few tumours also harboured some cytokeratin 18 (K18) positive cells. The expression of the stem cell marker CD44 was increased in the mammary ducts and tumours in the GLI1 positive mice. Lgr5 was expressed in the basal cell layer of the large mammary ducts as well as in the GLI1 induced tumours.
Conclusions: Mammary gland specific, long-term expression of GLI1 induces formation of different types of K5 and K6 positive tumours with basal character in transgenic mice. Induction of various types of tumours and expression of Lgr5 in the tumours as well as increased expression of the stem cell marker CD44 indicate that the expression of GLI1 affects mammary stem cells.

868 Genotoxicity/clastogenicity of ptaquiloside, the bracken (Pteridium aquilinum) carcinogen, towards human peripheral blood lymphocytes

R. Gil da Costa[1], P. Coelho[2], R. Sousa[3], M.M.S.M. Bastos[4], B. Porto[3], J.P. Teixeira[2], I. Malheiro[3], C. Lopes[1]. [1]*Instituto de Ciências Biomédicas Abel Salazar, Pathology and Molecular Immunology Department, Porto, Portugal,* [2]*National Institute of Health, Environmental Health Department, Porto, Portugal,* [3]*Instituto de Ciências Biomédicas Abel Salazar, Microscopy Department, Porto, Portugal,* [4]*Engineering Faculty – University of Porto, LEPAE – Chemistry Department, Porto, Portugal*

Background: Ptaquiloside (PTA), a bracken toxin, is a known carcinogen for animals but its implications on public health remain controversial (Yamada *et al*., 2007). This work addresses PTA's genotoxicity for human peripheral blood lymphocytes.
Material and Methods: PTA was isolated from bracken shoots collected in Ponte da Barca, Portugal, following methods by Ojika *et al*. (1985). Nuclear magnetic resonance techniques were used to confirm the compound's identity. The alkaline comet assay was performed according to Costa *et al.* (2008) on cells from 10 healthy donors exposed to 5 μg/ml PTA (or DMSO, negative control) in RPMI at 37ºC, for 5, 10, 20, 30, 40 or 50 min. Electrophoresis took place at 30 V for 20 min. Comet Assay IV (Perspective Instruments) software was used for slide analysis and for calculating tail intensity (TI). For chromosomal aberrations (CA) (5 donors) and sister-chromatid exchanges (SCE) tests (2 donors) cells were cultivated on supplemented RPMI. PTA was added at 24h (5, 10 or 20 μg/ml final dilutions). Bromodeoxyuridine was added to replicate cultures for SCE. Cells were then incubated for 48h and harvested after colcemid arrest. CA and SCE were counted on 100 and on 25 metaphases for each donor, respectively.
Results: The TI values for control/exposed cells at 5, 10, 20, 30, 40 and 50 min were 4.85/6.17, 5.11/5.75, 3.61/22.60, 4.77/28.53, 1.76/12.76, 1.62/10.52, respectively. Cytogenetic results were expressed, for controls and for each PTA dilution (5, 10 or 20 μg/ml) as the mean percentage of aneuploid cells (3, 15.3, 22.7, 46.4 respectively) and cells with chromosome/chromatid gaps and breaks (0.2, 2.4, 7.2, 14.5), mean number of gaps and breaks per 100 cells (0.2, 2.4, 7.8, 16.4), and the mean number of SCE per cell (9.4, 14.2, 18.4, 25.7).
Conclusions: The comet assay demonstrated that even PTA doses as low as 5 μg/ml are enough to induce DNA damage in a human *in vitro* model. Maximum damage was observed at 20−30 min, diminishing at 40−50 min, presumably due to DNA repair mechanisms. The cytogenetic tests show that at 48 h, despite such mechanisms, PTA originates structural and numeric CA and increased SCE in a dose-dependent manner. This suggests that PTA exerts its genotoxicity through multiple mechanisms and further support the hypothesis that PTA represents a significant threat to public health.

Reference(s)
Ojika *et al.* J Nat Prod 1985, 48: 634–637
Costa, *et al*. Toxicology 2008, 252: 40−48
Yamada *et al*. Nat Prod Rep 2007, 24: 798–813.

869 The lichen compound usnic acid disturbs mitochondrial function and induces autophagy in cancer cells

M. Bessadottir[1], M. Egilsson[1], E. Einarsdottir[2], G. Bjornsdottir[1], I.H. Magnusdottir[2], S. Omarsdottir[2], H.M. Ogmundsdottir[1]. [1]*University of Iceland, Faculty of Medicine, Reykjavík, Iceland,* [2]*University of Iceland, Faculty of Pharmaceutical Science, Reykjavík, Iceland*

Background: The lichen compound usnic acid (UA) is a component of the fat-burner Lipokinetix and has been shown to reduce ATP production in liver cell mitochondria. The effect on mitochondria can be directly related to the property of UA to shuttle protons across membranes. Autophagy is a process that can aid cell survival during nutrient shortage. UA inhibits the growth and proliferation of cancer cells but does not induce apoptosis.
Aims and Methods: To test for changes in inner membrane mitochondrial potential using JC-1 staining and measure levels of cellular ATP in UA-treated breast and pancreatic cancer cells. Also, to test if cells treated with UA showed signs of autophagy, using electron microscopy and immunostaining for the autophagosomal marker LC-3 and Western blotting for the autophagosomal cargo p62.
Results: A drop in inner membrane mitochondrial potential was demonstrated and reduced levels of ATP were observed in breast and pancreatic cancer cells treated with 5 μg/mL and 10 μg/mL of UA for 24 hours. Clear signs of autophagy were seen after treatment with UA, but results indicate that degradation of p62 does not occur. Therefore, in ongoing experiments we are testing for autophagosomal-lysosomal fusion and acidification using a tandem-tagged mRFP-GFP-LC3 fusion construct.
Conclusion: UA treatment of cancer cells causes a drop in mitochondrial membrane potential leading to reduced ATP production. This stimulates autophagy but apparently without degradation of autophagosomal content.

870 The lichen compound protolichesterinic acid affects lipid metabolism and induces ER stress in cancer cells

M. Bessadottir[1], E. Einarsdottir[2], G. Jonsdottir[2], S. Omarsdottir[2], H.M. Ogmundsdottir[1]. [1]*University of Iceland, Faculty of Medicine, Reykjavík, Iceland,* [2]*University of Iceland, Faculty of Pharmaceutical Science, Reykjavík, Iceland*

Background: The lichen metabolite protolichesterinic acid (PA) is a potent inhibitor of 5- and 12-lipoxygenase and has anti-proliferative effects on several types of cancer cells, as well as inducing apoptosis in multiple myeloma cells. Fatty acid synthase (FAS) is highly expressed in human carcinomas and appears to be required for their survival. The chemical structure of PA is very similar to known FAS inhibitors. Aims and methods: To test if PA inhibited FAS by measuring uptake of ^{14}C-acetate into cells and to test for ER-stress, which is a known consequence of FAS inhibition, using Western blotting for phosphorylated-eIF2α. Signalling through major stimulatory pathways was tested by measuring activation of ERK1/2 and STAT3.
Results: Uptake of ^{14}C-acetate into breast cancer cells was reduced in a dose-depedent manner by PA reaching 33% at 10 μg/mL. The same concentration