on DDSB response as the depletion of wtBRCA1. Contrary to wtBRCA1-downregulation affecting both HR and NHEJ repair, the expression of BRCA1Δ14−15 and Δ17−19 variants showed predominant NHEJ impairment. We conclude that both variants negatively affect genomic stability but solely BRCA1Δ17−19 influences the clonogenic potential in MCF-7 cells.
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419 USER-based Approach for Identification of BRCA1 Alternative Splicing Variants

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Background: BRCA1 is a multifunctional protein with a key role in regulation of DNA double strand break repair. Alterations in the *BRCA1* gene are responsible for breast/ovarian cancer susceptibility. Except for the aberrant splicing emerged from the *BRCA1* gene alterations, endogenously expressed alternative splicing variants (ASVs) have been described in various cell lines and tissues. Some of these represent in-frame ASVs that can be translated into the protein isoforms lacking crucial functional domains of BRCA1 coded by spliced exons. With a growing need to understand the significance and function of BRCA1 and its isoforms, a robust tool for identification of BRCA1 ASVs is required since their catalogue has not been established yet. Here we present an approach for the identification of BRCA1 ASVs.
Material and Method: cDNA from MCF-7 cell line was amplified with uracil-containing specific primers and cloned into the pNEB206A vector using uracil-specific excision reagent (USER Friendly Cloning Kit; NEB). Created constructs were used for transformation of TOP10 *E. coli* competent cells. Colonies selected using the IPTG/X-Gal screening were cultivated in 96-well plate for 4 h and used for direct PCR screening with BRCA1-specific primers and subsequent sequencing.
Results and Discussion: We identified 28 individual ASVs of BRCA1 mRNA in MCF-7 cells. Ten in-frame ASVs lacked exons coding for functionally important BRCA1 domains including the DNA-interaction domain (Δ10b), nuclear localization signal (Δ9) or RING-finger domain (Δ3−6). These variants may have a critical impact on resulting BRCA1 function. Eleven ASVs exhibited frame-shift leading to premature termination of translation and 7 ASVs with large deletions (Δ2−14 and more) lacked exon 2 containing the initiation codon. Therefore, the translation of these ASVs was considered improbable.
Since BRCA1 mRNA occurs (in individual cell) in a number of ASVs which differ also in short (3−21 bp) in-frame deletions, the identification of such complex splicing pattern by gel electrophoresis and subsequent sequencing is not possible. The use of next-gen transcriptome analysis is also not suitable due to the presence of the large exon 10a (3426bp) and ASVs with complex exonic composition (e.g. Δ4+Δ8−9+14b).
Conclusion: The USER-based cloning of individual mRNA variants represents robust and reliable system for identification of complex BRCA1 mRNA splicing.
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420 A Platform of Porous Biomaterials as 3D Culture Systems for Cancer Biology

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Background: The role of stem cells in tissue development and repair is beginning to be unravelled and will open remarkable opportunities to improve current medical treatments. Yet, the cascade of events that enable us to distinguish between abnormal and functional tissue morphogenesis is not well known and the potential involvement of stem cells in cancer initiation or tissue regeneration is still at an embryonic stage. Most biological studies rely on culturing cells onto two-dimensional (2D) substrates, which poorly reflect the three-dimensional (3D) environment that governs the physical, chemical, and biological processes at the heart of tissue development. Here, we introduce a library of 3D culture systems − scaffolds − with enhanced cell-material interactions.
Materials and Methods: 3D scaffolds made of biodegradable synthetic polymers were fabricated by either rapid prototyping, eletrospinning and their combination. Mesenchymal stem cells derived from bone marrow were isolated from patients after informed consent, seeded on the scaffolds and cultured for up to 35 days. Cell morphology was observed by scanning electron microscopy. Cell number and metabolic activity were quantified by DNA and alamar blue assays. Differentiation was assessed by gene expression, while extrfacellular matrix (ECM) formation by biochemical assays.
Results: 3D scaffolds with tailored mechanical and physichochemical properties could be fabricated by different processing technologies. While rapid prototyping resulted in the fabrication of scaffolds with controlled porosity at the macro scale, electrospinning enabled the creation of fibrillar meshes mimicking the physical micro and nano scale dimensions of native ECM. Nutrient availability had a profound effect on tissue formation in 2D and 3D. Despite steep nutrient concentration gradients in 3D scaffolds, stem cells proliferated while avoiding significant death. Cell migration into millimeter-size circular patterns in the scaffold's pores was supported by ECM organization. Higher concentrations of nutrients controlled the rates of proliferation and did not induce differentiation markers. Furthermore, scaffolds with customized physicochemical and surface properties influenced stem cell morphology and activity.
Conclusions: These 3D scaffolds offer a new platform to study the mechanisms behind stem cell driven tissue morphogenesis and may play a role in cancer biology research to create organotypic 3D models to study cancer initiation and development, as well as the potential involvement of stem cells in these processes.

421 Ethanol Directly Modulates Reactive Oxygen Species Generation in Oral Squamous Cell Carcinoma Cell Lines

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Background: Oral cancer is the sixth most common cancer in the world and has one of the highest death rates, due to it being largely asymptomatic until the latter stages of the disease. It is a major worldwide public health problems with the incidence and mortality rising in several regions of the world, including Europe, South Central Asia and Australia. Smoking poses the highest risk for oral cancer followed closely by the consumption of alcohol. This study aims to further understand the role of ethanol in the carcinogenic transformation of oral tissue with the specific interest in reactive oxygen species (ROS) production and the effect on cell cycle progression in oral cavity squamous cell carcinoma cell lines.
Materials and Methods: Two oral cancer cell lines were utilised as models for this study, Ca9.22 (gingival) cell line and TR146 (buccal mucosa) cell line. Cells were cultured in the presence or absence of a range of ethanol concentrations over 6, 24 and 48h time points. Cell viability was determined using Alamar Blue. Alcohol dehydrogenase assays were performed to monitor the activity of the enzyme within the cell and to ensure the absence of the enzyme in the cell culture medium. Extracellular production of hydrogen peroxide (H_2O_2) was monitored using Amplex Red and intracellular production of H_2O_2 was monitored using 2′,7′-dichlorfluoresceindiacetate (DCFH-DA) and analysed by both flow cytometry and confocal microscopy. Perturbations in the cell cycle were analysed with flow cytometry. Expression levels of proteins that control the cell cycle were determined by western blotting. The levels of expression of specific genes underlying the control of the hypoxic response in the cell were determined by quantitative real time polymerase chain reaction.
Results: Ethanol concentrations from 0.1−3% showed no detrimental effect on cell survival over the given time course ($n = 8$). The presence of ethanol was found to reduce the release of H_2O_2 in a dose dependent manner ($p < 0.5$–0.001 in relation to control, $n = 3$) however when normalized for cell numbers over the duration of the study a biphasic response was observed. Cell cycle analysis revealed a biphasic trend at the lower concentrations of ethanol with no significant reduction in the proliferation index at the time points examined. No significant induction of apoptosis or polyploidy events were observed.
Conclusion: These findings suggest that the significant reduction in the production of cellular H_2O_2 is as a result of ethanol and not its first metabolite acetaldehyde, which is a known carcinogen. Lack of induction of apoptosis may allow for both low levels of ROS and ethanol to cause progressive damage to DNA. This alteration in oxidative stress could induce a potential switch from apoptotic to necrotic cell death which may lead to eventual carcinogenic transformation.

422 Investigation of the Apoptotic Pathways Activated by Equol and Tamoxifen in MCF-7 Breast Cancer Cells

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Background: Soy phytoestrogens, such as daidzein and its metabolite equol, have been proposed to be responsible for the low breast cancer rate in Asian women. However, since these compounds possess both estrogenic and anti-estrogenic properties, it remains unknown whether they provide protection against tumor development and progression. Since the majority of estrogen receptor positive (ER+) breast cancer patients are treated with the selective estrogen receptor modulator tamoxifen, the basic objective of this project is to determine whether equol enhances or inhibits tamoxifen's anti-tumor effect and to identify the molecular mechanisms involved. For this purpose, we examined the individual and combined effects of equol and tamoxifen on the ER+ breast cancer cell line MCF-7.
Materials and Methods: In order to examine the effects of equol and tamoxifen on the MCF-7 cells, we used viability assays (MTT assay), cell cycle analysis, annexin-V/PI staining, cell death ELISA, and western blot