wax using a Leica ASP or Pelorus system with >1 hour formalin fixation. Sections of these were then cut at 10 microns and four sections pooled for DNA extraction, performed using a Ambion Recoverall™ total nucleic acid isolation kit optimised for FFPE samples. Quantitiative PCR detection of mutations present was performed using the ARMS Scorpions kit (DxS/Qiagen, Manchester, UK) according to the manufacturer's instructions with an AB7500 PCR machine (Applied Biosystems Inc, Foster City, CA).
Results: Sufficient DNA recovery was obtained using both processing methods, despite the higher temperature and xylene free processing used by the Pelorus, though slightly higher recovery was observed using the ASP processor. Histology of the cell line pellets is feasible and routinely performed for each pellet, and the results can be rapidly compared with response to drugs or candidate molecules in vitro.
Conclusion: Cell lines containing specific integrated mutations provide an ideal alternative to tumour samples for target validation and drug discovery, to which one can also now add companion diagnostic design, initial validation, and quality assurance.

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Identification of biomarkers associated with tumor progression using laser microdissected tissues from colon adenoma and cancer

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Background: Colorectal (CRC) malignancies rank worldwide at third place for tumor diseases and account for an annual mortality rate of 492 000 cases. Although several molecular events are known to be involved in the transition from normal tissue to adenoma and finally to undifferentiated carcinoma it remains a challenge to discover new and more reliable biomarkers for diagnosis, prognosis and prediction of outcome. Towards this end a study was designed to identify potential biomarkers which are associated with the molecular events leading from epithelial adenoma to the early stages of carcinoma.
Material and Methods: A new biomarker discovery strategy was developed to combine the cell specificity and the selectivity of laser capture microdissection (LCM) with the resolution power and sensitivity of liquid-chromatography (LC)-matrix-assisted-laser-desorption/ionization mass spectrometry (LC-MALDI-MS). We carefully selected a group of closely matched patients (n = 10 for each group) afflicted with epithelial adenoma (high dysplasia) or early stages of carcinoma (stage I) and used the derived normal as well as the matched tumor tissue samples to reveal protein expression differences. According to this LC-MALDI-MS strategy microdissected cells were lysed and extracted proteins were digested with trypsin. Obtained peptides were separated by capillary reversed phase HPLC (Agilent). The resulting LC-fractions (300) were spotted on prespotted AnchorChip targets (PAC, Bruker) and tryptic fragments subsequently detected by reflector MALDI-MS (ultraflex III,Bruker) measurements. Differential peptide analysis was performed to discover robust and significant expression differences between patient groups. Therefore, only m/z ions displaying a minimum twofold difference and a p-value of 0.01 between groups were considered for further analysis. The selected peptides were subsequently fragmented by MS/MS experiments to reveal their primary sequence and protein identity.
Results: Up to 7000 ion signals ranging from m/z 800 to 4000 were generated and used for statistical analysis. Our targeted biomarker discovery approach resulted in the identification of more than 30 biomarker candidates.
Conclusion: The identified biomarker candidates are involved in diverse cellular functions and can now probably be linked to early or late events of tumor progression. Currently these biomarkers are being validated using antibody based assays to further analyze their potential as markers in a clinical setting.

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Detection of miR-302, an ES-specific microRNA, in cancer cell lines and tissues

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Background: The miR302–367 is a cluster of microRNAs that are exclusively expressed at high levels in embryonic stem (ES) cells. Indeed, miR-302–367 cluster may play an essential role in maintaining hESC pluripotency and self-renewal. In addition, promoter of miR302–367 is transcriptionally regulated by the ES cell master regulators, e.g. Oct3/4, Sox2, and Nanog (OSN). Previous studies indicated that expression of OSN could be detected in tumor samples. Therefore, expression of miR302s as a downstream component of OSN is also conceivable in the cancer cells and has the merit of being studied as a potent tumor marker.
Material and Methods: Generally, amplification and detection of microRNAs by PCR is not straightforward, due to their short lengths (20−24nt). In addition, high similarity of miR302-family members, make it difficult to specifically detect individual members. In the current study, a stretch of A-nucleotides were added to the 3′-end of the extracted RNAs by using poly-A polymerase. cDNA was then synthesized using an oligo-dT primer that was anchored to a tag sequence on its 5′-end. The tag could be used as a reverse primer in the subsequent stages. Additionally, the forward primer was selected such that it could specifically amplify miR302b.
Results: Specificity of the PCR was examined using a vector containing miR-302a, miR-302c, and miR-302d but not miR-302b. Based on our data, the miR302b-PCR system was specific, at least in the presence of 6×10^6 copies of the vector in 45 cycles. Consequently, the system was evaluated in different tumor samples and cell lines. Interestingly, the expression of miR302b was detected in some brain and bladder tumor cell lines in addition to tumor samples.
Conclusion: Recently, we have shown the functionality of miR302–367 promoter in a rare sub-population of brain tumor cell lines. The results of the current study also demonstrated a low expression level of miR-302 in cancer cells. These results may indicate that upstream regulators of miR-302, namely OSN, are expressed in a rare sub-population of cancer cells. In other words, an ES-like expression pattern and stemness properties may exist in this sub-population of putative cancer stem cells.

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Ex vivo assay to monitor response to chemotherapeutic agents in plucked human hairs

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Background: Plucked hair is a valuable surrogate biomarker tissue to monitor pharmacodynamic (PD) responses. Hair collection is also minimally invasive, simple and permits frequent sampling. We have previously developed immunohistochemistry (IHC) labeling protocols for plucked human scalp hair, enabling target response to treatment to be monitored directly. We now present an assay for the *ex vivo* maintenance, treatment and labeling of human hair. This provides effective proof of concept data for the detection in hair of a target protein response, before progressing to a clinical trial.
Methods: Donor hairs were plucked and immediately transferred to a maintenance medium containing vehicle or 200 nM Tarceva for 2 or 10 mins before being harvested and fixed (5 hairs per donor, per treatment) along with freshly plucked untreated hairs (5 per donor). Hairs were then embedded in wax, sectioned and IHC labeled for phospho-ERK1/2 (p-ERK1/2) using methods previously developed. The amount of p-ERK1/2 present was quantified using the Ariol® slide scanning system.
Results: In fresh hairs the p-ERK1/2 was restricted to distinct bands across the outer root sheath (ORS) of the hair. In control hairs maintained *ex vivo*, there was some diffusion of the banded labeling after 2 mins and complete diffusion after 10 mins, with labeling then presenting throughout the ORS. Labeling in hairs treated for 2 mins with Tarceva was similar to that of hairs treated with vehicle for 2 mins, whilst labeling was greatly reduced in hairs treated for 10 mins with Tarceva compared to 10 min vehicle treated hairs. Labeling was quantified on the Ariol® scanning system and after 10 min treatment with Tarceva a significant 73%, 59% and 61% decrease in donor 1, 2 and 3, respectively, was observed compared to 10 min vehicle treated hairs (Students *t*-test: donor 1 p = 0.0002, donor 2 p = 0.01, donor 3 p = 0.007).
Conclusion: This preliminary data indicates good reproducibility in the *ex vivo* hair assay and a significant reduction in p-ERK1/2 levels following 10 min Tarceva treatment. The *ex vivo* hair assay provides valuable proof of concept biomarker data, prior to collection of hairs within a clinical study, confirming whether the hairs are suitable surrogates for the target protein of interest.

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Epidermal growth factor receptor (EGFR) gene amplification is not the cause of protein overexpression in penile carcinoma

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Background: Squamous cell carcinoma of the penis affects mainly people with poor hygiene habits in undeveloped countries. Epidermal growth factor receptor (EGFR) is a well characterized tyrosine-kinase receptor that has