from paraffin embedded specimens were also examined for possible mutations. All PCR reactions were run in triplicates.
Results: In cell lines, mutations were identified without ambiguity up to the 0.5% dilution. No positive samples were found in non-tumour tissues. This first analytic part allowed us to define for each mutation proper Cq cut-off to analyse KRAS mutation in specimens. The results obtained by castPCR for the 24 tumours were concordant to those previously reported by three different methods (HRM-sequencing, snapshot and TaqMan probes).
Conclusion: This work shows different validation steps of a new KRAS genotyping technology, suitable for KRAS determination in the clinics on paraffin embedded specimens with a very good sensibility and specificity. It is a quick, one-step technology compatible with diagnosis demand. Testing is on going using the 7500 Dx Fast that is to be CE IVD marked. The assessment of this highly sensitive technology will be carried out on body fluids to analyse its accuracy for KRAS testing when tumour tissues are not available.

182 Clinical significance of target genes of Wnt/beta-catenin pathway in hepatocellular carcinoma

E.K. Tung[1], C.K. Mak[1], I.O. Ng[1]. [1]*The University of Hong Kong, Pathology, Pokfulam, Hong Kong*

Background: Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and is particularly prevalent in Southeast Asia and China including Hong Kong. Wnt/beta-catenin pathway is one of the important signaling pathways and therapeutic target in liver cancer. In this study, we aimed to reveal the expression profile of these target genes and to investigate their clinicopathological significance in human HCC.
Material and Methods: To elucidate the molecular mechanism of the deregulation of Wnt/beta-catenin pathway in HCC, we performed high-throughput quantitative RT-PCR analysis (Low Density Microarray, LDA) to study the gene expression patterns of Wnt-signaling molecules, include Wnt ligands, Fizzled receptor proteins, Wnt-related genes, on 38 pairs of human HCC samples and their corresponding non-tumourous livers. Ten target genes of Wnt/beta-catenin signaling were also evaluated, include c-myc, cyclinD1, LEF1, c-jun, Axin-2, VEGFA, DKK1, Frizzled 7, Twist1 and EGFR.
Results: LEF1 and DKK1 were found to be significantly overexpressed in human HCC (63% and 66%, respectively) when compared with their corresponding non-tumourous livers ($P < 0.001$ and $P = 0.005$, respectively). In addition, the expression level of LEF1 and DKK1 positively correlated with one another ($P = 0.037$). LEF1 was found to significantly correlate with venous invasion, absence of encapsulation and advanced tumour stage ($P = 0.043$, 0.008 and 0.045, respectively). The combination of LEF1 and DKK1 further increased their correlation with clinicopathological parameters in human HCC, like venous invasion and poorer cellular differentiation (Edmondson grading) ($P = 0.021$ and 0.047, respectively).
Conclusions: Both LEF1 and DKK1 are upregulated in human HCC and associated with more aggressive tumour behaviour.
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183 Correlated expression analysis of VEGF family members and lipid inflammatory mediators in human colon polyps and carcinomas and liver metastases

S. Pringels[1], N. Van Damme[2], M. Peeters[2], J. Grooten[1]. [1]*Ghent University, Department of Biomedical Molecular Biology, Gent, Belgium,* [2]*Ghent University Hospital, Department of HepatoGastroenterology, Gent, Belgium*

Background: Inflammatory mediators, such as prostaglandin E_2 (PGE_2), and responsive angiogenic factors, mainly vascular endothelial growth factor A (VEGF-A), have emerged as pathways driving neo-angiogenesis and supporting the progression and metastasis of solid tumours.
Materials and Methods: To understand the relation in human solid tumours between COX and LOX-derived eicosanoids and expression of VEGF family members ($VEGF^F$) (VEGF-A, -B, -C, -D and PlGF), we performed a RT-qPCR comparative expression analysis of colon carcinoma samples. Considering shifts in expression profiles during tumour progression, a similar analysis was done for colon polyps and liver metastases.
Results: Up to now, tumour samples and matched normal colon tissues from 52 patients were analyzed. The results showed a complex and diversified expression phenotype. 88% of the tumour samples showed increased expression of at least one VEGF family member. In a considerable proportion of samples multiple VEGF family members were overexpressed with a predominance of VEGF-A and especially PlGF. Correlating the $VEGF^F$ and eicosanoid enzymes gene expression profiles not only revealed a clear linkage between both signaling pathways but also a clear association of COX2 with VEGF-A, VEGF-C and PlGF.
A similar analysis was performed on 23 colon polyps and 30 liver metastases. Strikingly, already in polyps a pronounced inflammatory expression profile with increased expression of COX enzymes was apparent. This was accompanied by an increased expression of mainly VEGF-A and PlGF. Also in liver metastases, an inflammatory signature accompanied by $VEGF^F$ expression was apparent. Remarkably, the $VEGF^F$ profiles observed in liver metastases were near indistinguishable to those from the primary colon carcinomas. Yet, the eicosanoid enzymes showed differential expression profiles which may be due to the different tumour microenvironment in the liver.
Conclusions: The results from this correlated expression analysis of VEGF family members and genes involved in eicosanoid biosynthesis are promising for the diagnosis and prediction of treatment outcome of colon cancer patients. In addition, our results clearly indicate that the perception of a $COX2/PGE_2$-driven VEGF-A expression, sustaining neo-angiogenesis, is an oversimplification.

184 Diagnostic radiation exposures among children diagnosed with leukemia vs. solid tumours in US, UK and Germany, 1995–2005

R. Rohrer[1]. [1]*University of Pittsburgh, Epidemiology, Pittsburgh, USA*

Background: Determining the role of medical diagnostic radiation exposures in children subsrequently diagnosed with a type of cancer is challenging and even controversial. Recent literature reviews point to these difficulties in case studies and sample size due to the rarity of childhood cancers in general. This study will start with a literature review of both diagnostic x-rays and CT scans over a ten year period in the US, UK and Germany to determine both national differences and differences in frequency among children diagnosed with leukemia versus solid tumours.
Material and Methods: The author will use published sources, archival materials from Children's Oncology Group and parental questionnaire.to determine pre-natal, in utero and childhood exposures.
Results: Preliminary results indicate a higher frequency among children diagnosed with any type of leukemia, followed secondly by children with central nervous system tumours. Further studies of each major tyoe of sarcoma would be useful to further study.
Conclusions: This study found that children with leukemias were more likely to have a history of prenatal exposure to diagnostic radiation in the western nations studied over this time period. Much further and detailed study is warranted by time period and by cancer type.

185 Imaging by confocal endomicroscopy: new insight for in vivo tissue diagnosis of head and neck cancer

M. Abbaci[1], O. Casiraghi[2], S. Temam[3], J. Bosq[2], C. Laplace-Builhé[1]. [1]*Institut Gustave Roussy, Platform of Cellular Imagery and Cytometry, Villejuif, France,* [2]*Institut Gustave Roussy, Department of Pathology, Villejuif, France,* [3]*Institut Gustave Roussy, Department of Otorhinolaryngology and Head and Neck Surgery, Villejuif, France*

Background: Histological analysis of tissues is essential for cancer diagnosis and to guide therapeutic choices, but a biopsy is an invasive procedure that may delay treatment decisions. The biopsies required for the diagnostic may also cause changes in local tissue and alter the assessment of small tumour extension during the resection procedure. Recently, non-invasive optical technologies based on the miniaturization of imaging systems have been proposed to achieve in vivo "optical biopsies". Among them, fibered confocal endomicroscopy (CEM) provide dynamic images of the microarchitecture of tissues during a conventional endoscopy. In this study, we evaluated the potential of CEM to aid the detection of precancerous lesions and laryngeal cancer.
Material and Methods: Fluorescent agents clinically approved or in clinical trials were used to enhance image contrast. A staining protocol directly transferable to humans has been developed for a futur clinical study. 47 non cancerous and cancerous tissue samples were taken from human surgical head and neck specimens after total or partial surgery. After topical application of acriflavine hydrochloride and sodium fluorescein, samples were imaged with CEM (Cellvizio, MKT), and conventional confocal microscope (Leica SPE). An histomorphologic correlation study was subsequently performed on 140 CEM images, 140 confocal images and 47 conventional histological preparations of the same samples. The images were interpreted by two pathologists in a double-blind trial.
Results: The 2 fluorescent dyes allowed a morphological analysis based on the cellular distribution, detection of nuclear abnormalities and visualization of disorders of keratinization. The histological diagnostic such as dysplasia or invasive squamous cell carcinoma could be interpreted with the images from CEM, although the image quality was inferior to conventional confocal microscopy. Statistical correlation with conventional histology is being finalized.
Conlusions: This study demonstrated the CEM ability to provide "histological-like images" that can be interpreted by pathologists, even on complex tissues as in the case of head and neck cancers. The study conducted jointly with clinicians and pathologists also identified the conditions of transfer of this