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PO08
EWSR1: Identification and functional characterization of a novel target gene locus in lymph syndrome

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Background. Lymph syndrome represents the most common, autosomally inherited cancer predisposition worldwide. MMR deficiency results in microsatellite instability (MSI), i.e. genome-wide accumulation of somatic alterations at repetitive DNA sequence motifs. MSI, used as a diagnostic tool to identify HNPPC-related CRCs, is considered to be a major cause of colorectal cancer. In search for novel target gene loci we identified a putative polymorphic marker (C767G) in the 3’UTR of the EWSR1 gene.

Methods. We determined the length of the EWSR1 3’UTR tract motif, (T)16, by PCR and fragment analysis of 92 HNPPC-related CRCs (MLH1: n=50, MSH2: n=28, MSH1 promoter methylation: n=14), 8 sporadic CRCs, 64 sporadic gastric cancers, 35 sporadic CRCs and 4 cell lines. EWS protein expression was assessed by immunohistochemistry on a tissue microarray and immunoreactivity was scored semiquantitatively.

Results. HNPPC-related CRC (n=2), sporadic CRC (n=1), sporadic gastric (n=2) and mast cell cancer (n=0) were shown to have an allele with C767G in the 3’UTR, but none (n=52) of the sporadic MSS CRCs and sporadic MSS gastric cancer (n=3). IHC showed significant downregulation of EWS expression in sporadic CRCs (p=0.005), but no difference in HNPPC CRCs (p=0.7). Since the (T)16 tract may represent a binding site for an RNA element binding protein, it could have an effect on EWSR1 mRNA stability/translation.

Conclusion. The (T)16 tract in the 3’UTR of the EWSR1 gene represents a novel target gene locus in lymph syndrome allowing for highly sensitive and specific identification of MMR-deficient CRCs. IHC suggests a regulatory role of this locus on EWS protein expression in HNPPC CRCs.

PO09
Expression of CD44 isoforms and WNT pathway in colorectal carcinogenesis: from mouse to man

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Background. Sporadic colorectal carcinomas (CRCs) mostly exhibit mutations of the adenomatous polyposis coli gene (APC). ApoMin+ mice are prone to polyposis formation. The surface protein CD44, particularly its variant isoforms CD44v6/7, have prognostic value in CRC. Our aim is to evaluate potential interactions between the APC gene product and CD44v6/7 and their downstream signal transduction cascades.

Methods. C57BL/6J ApoMin+ or CD44v6+/+(group 1) and ApoMin+/ +CD44v6/7—/—(group 2) mice were generated and microarrays of polypos vs. non-polypos regions were performed. Based on this survey, immunohistochemistry was assessed for expression of β-catenin, E-cadherin, CD44v6/7, CD44v6, CD44v6/10, active caspase-3, FAK and pSTAT3 in mouse polypos. Moreover, a tissue microarray (TMA) with 1200, non-consecutive primary human colorectal cancers was evaluated for E-cadherin, CD44v6/7, CD44v6 and FAK.

Results. Absence of CD44v6/7 in ApoMin+ mice reduced polypos formation and increased the survival rates strongly. Microarray gene profiling showed the upregulation/downregulation of selected genes involved in WNT/β-catenin, E-cadhe-

PO10
KRAS and BRAF mutations predict primary resistance to Imatinib in gastrointestinal stromal tumors (GIST)

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Background. Gastrointestinal stromal tumors (GIST) are characterized by gain-of-function mutations in KIT/PDGFRA genes leading to a constitutive receptor activation which is well counteracted by Imatinib. However, cases in which Imatinib as first line treatment has no effects are reported (primary resistance). Our purpose is to investigate alternate mechanisms of resistance in sporadic tumors, not reported so far in mutated GIST, possibly explaining the primary resistance to targeted treatments.

Methods. Experimental design. Two independent naïve GIST cohorts have been analyzed for KIT, PDGFRA, KRAS and BRAF mutations by direct sequencing. Cell lines expressing a constitutively activated receptor were treated with Imatinib, followed by KIT, PDGFRA, KRAS and BRAF sequencing to find the cause of resistance.

Results. In naïve GISTs carrying activating mutations in KIT or PDGFRA a concomitant activating mutation was detected in KRAS (5%) or BRAF (about 2%) genes. In vitro experiments demonstrated that Imatinib was able to switch off the mutated receptor KIT but not the downstream signalling trigger by RAS-RAF effectors. These data suggest the activation of MAPK pathway as a possible novel mechanism of primary resistance to Imatinib in GISTS and could explain the survival curves obtained from several clinical studies where 2-4% of GIST patients treated with Imatinib, despite carrying KIT sensitive mutations, do not respond to the treatment.
Methods. QoCC indicators will be defined through up-to-date literature following the Delphi process, and will refer to all incident cancers of colon-rectum, prostate and ovary/t uterus occurring between 2011 and 2013 in Canton Ticino.

Results. An extract of the pilot study results concerning 428 colorectal cancers (301 colon, 127 rectum) is the following: proportion of patients with microscopic diagnosis (colon=96.7%; rectum=100%) and with defined tumour histotype according to WHO in the biopsy/surgical resection (colon=97.6%; rectum=100%); proportion of surgical patients (within 6 months since diagnosis) (colon=87.5%; rectum=67.2%); proportion of patients with defined tumour site in the biopsy/surgical resection (colon=93.3%; rectum=89.6%); proportion of surgical patients with known resection margins (colon=95.2%; rectum=95.2%) and with lymphadenectomy (colon=99.3%; rectum=96.4%); proportion of surgical patients not undergoing neo-adjuvant therapy with more than 12 lymph nodes examined (colon=84.4%; rectum=84.1%); mean number of examined lymph nodes in surgical patients not undergoing neo-adjuvant therapy (colon=18.2±8.3; rectum=16.6±7.2); time (days) from biopsy to surgery in surgical patients not undergoing neo-adjuvant therapy (colon=15.2±18.1; rectum=27.4±36.2).

Conclusions. The QoCC approach, based on up-to-date incidence years, will allow a quick translation of results into the daily clinical practice, will favour the process of standardization of care, based on the evidence-based medicine and will create a comparable platform for other Cancer Registry initiatives.

PO12 Glypican-3 expression in non-tumoral liver samples
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Background. Glypican-3 is overexpressed in most hepatocellular carcinoma (HCC) and used as a diagnostic marker of HCC. Recently, Glypican-3 has been shown to be expressed in liver tissue of chronic hepatitis C virus (HCV) infection. This study was aimed to investigate Glypican-3 expression as a predictive marker for development of HCC in patients with chronic HCV infection.

Methods. Needle biopsies of non-tumoral liver tissue of chronic HCV patients with contemporary HCC (n=21) were stained for Glypican-3 expression by immunohistochemistry. Similarly, needle biopsies of liver tissue of chronic HCV patients, that did not develop a HCC for following 5 years (n=21) and were non-responders to therapy were stained for Glypican-3. Using a 0–3+ scale, the staining was described in both groups as 0 staining (negative), 1+ staining (<10% of cells), 2+ staining (10–25% of cells), or 3+ staining (>25% of cells). All liver samples had severe fibrosis (F9) or cirrhosis (F9).

Results. Liver biopsies were divided into three groups according to activity (METAVIR score A1-A3). Mean score of Glypican-3 expression in non-tumoral tissue of chronic HCV infected patients with contemporary HCC was 0.2 for A1, 1.1 for A2 and 1.6 for A3. Chronically infected HCV patients with A1 had a score of 0.25, A2 of 0.8, and A3 of 1.2.

Conclusions. There was a trend of non-tumoral tissue from chronic HCV infected patients with contemporary HCC towards a higher Glypican-3 expression. However, no statistically significant difference was observed between the two groups. Glypican-3 expression correlated better with the grade of activity.

Tumor – Pathology, miscellaneous

PO13 DNA methylation of the homeobox genes PITX2 and SHOX2 predicts outcome in non small cell lung cancer patients
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Background. Biomarkers that facilitate prediction of disease progression in lung cancer patients might be clinically valuable in optimizing individualized therapy. In this study, the ability of DNA methylation biomarkers PITX2 and SHOX2 to predict disease outcome in lung cancer patients was evaluated.

Methods. Quantitative, methylation-specific (HeavyMethyTMT) real-time PCR assays were used to measure DNA methylation of PITX2 and SHOX2 in bisulfite-converted DNA from formalin-fixed, paraffin-embedded tissues from 474 non-small cell lung cancer patients.

Results. In univariate Cox's Proportional Hazard analysis, SHOX2 and PITX2 hypomethylation were significant predictors for disease progression (SHOX2: n=465, HR=1.395 [1.330–1.463], p=0.002; PITX2: n=445, HR=1.312 [1.059–1.623], p=0.003). Patients with hypomethylation of either PITX2 and/or SHOX2 (n=310) showed a significantly higher risk of disease progression as compared to patients with hypermethylation of both genes (n=166; HR=1.555 [1.210–1.999], p=0.001). This was particularly true for the subgroup of patients receiving no adjuvant radio- or chemotherapy (n=258, HR=1.838 [1.522–2.209], p=0.002). In multivariate analysis, both biomarkers added significant independent prognostic information to pT, pN, pM, and grade. In addition, SHOX2 and PITX2 DNA methylation were shown to be inversely correlated with TTF1 (also known as NKX2.1) expression (PITX2: p=0.018, SHOX2: p<0.001).

Conclusions. DNA methylation of PITX2 and SHOX2 are independent prognostic biomarkers for disease progression in non-small cell lung cancer patients.

PO14 Impact of the new immunohistochemical panel for the diagnosis of lung cancer on overall patients outcome
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Background. The availability of targeted therapies created the need for precise subtyping of lung cancers. Aim of the study is to analyse the impact of the new immunohistochemical diagnostic panel on incidence and survival of lung cancer patients by histotypes.

Methods. Patients were selected from the Ticino Cancer Registry and categorized into the four WHO-defined histotypes: 2-year overall survival (OS) was performed for patients with a 24-month complete follow-up (incidence period 1996–2008). Trend analysis of survival probability was computed.

Results. 2,467 cancer patients were selected: 40.4% adenocarcinoma; 21.2% large cell carcinoma, 15.3% small cell carcinoma, 23.8% squamous cell carcinoma. We observed an increasing trend of 2-year OS of all cases, an improved and decreased OS in SqCC and LCC, respectively.

Conclusions. The introduction of an immunohistochemical panel could have influenced not only the incidence of different lung cancer subtypes, but also the short-term outcome of patients, raising the need for