PO08
EWSR1: Identification and functional characterization of a novel target gene locus in Lynch syndrome
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Background. Lynch 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. In search for novel target gene loci we identified a poly-T tract (T)16 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=90; MSH2: n=8; MLH1 promoter methylated: n=14), 8 HNPPC-related gastric cancers, 64 sporadic gastric cancers, 35 sporadic CRCs and 4 cell lines. EWS protein expression was assessed by immunohistochemistry on a tissue microarray and immunoreactivity scoring semiquantitatively.
Results. HNPPC-related CRC (78/79), patients with MLH1 promoter methylated (14/14), sporadic MSI CRC (12/12), HNPPC-related gastric cancer (8/8) and sporadic MSI gastric cancer (10/15) shown novel alleles in 3’UTR, but none (0/13) of sporadic MSS CRC and sporadic MSS gastric cancer (0/4). 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 AU-rich element binding proteins 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 Lynch 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). ApcMin/+ mice are prone to polyp formation. The surface protein CD44, particularly its variant isoforms CD44v6/v7 have prognostic value in CRC. Our aim is to evaluate potential interactions between the APC gene product and CD44v6/v7 and their downstream signal transduction cascades.
Methods. C57BL/6j ApcMin/+CD44v6v7/+ (group 1) and ApcMin/+CD44v6v7/- (group 2) mice were generated and microarrays of poyps vs. non-polypos regions were performed. Based on this survey, immunohistochemistry was assessed for expression of β-catenin, E-cadherin, CD44pan, CD44v6, CD44v9/10, active caspase-3, FAK and pSTAT3 in mouse polyps. Moreover, a tissue microarray (TMA) with 1420, non-consecutive primary human colorectal cancers was evaluated for E-cadherin, CD44v7, CD44v9 and FAK.
Results. Absence of CD44v6v7 in ApcMin/+ mice reduced polyp formation and increased the survival rates strongly. Microarray gene profiling showed the upregulation/downregulation of selected genes involved in WNT/PI3K pathways. Expression of β-catenin, E-cadherin, CD44pan and pSTAT3 was significantly different (0.0001<p<0.04) between polyps and normal mucosa. Protein expression of β-catenin, CD44pan and CD44v9/10 differed significantly in adenomas between genotype groups. In TMA, E-cadherin expression was positively related to CD44v9. Loss of both markers was associated with poor prognosis (p<0.01).
Conclusion. These results exhibit the possible role of CD44 isoforms in CRC pathogenesis by interaction with the WNT pathway and support their use as prognostic markers mostly in advanced stages.

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 alterations in downstream effectors, 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 and Imatinib responding KIT, alone or in combination with activated KRAS and BRAF, were produced and treated with Imatinib. KIT receptor and its downstream effectors were analyzed by direct Western blotting.
Results. In naïve GISTs carrying activating mutations in KIT or PDGFRA a concomitant activating mutation was detected in KRAS (56%) or BRAF (about 28%) genes. In vitro experiments demonstrated that Imatinib was able to switch off the mutated receptor KIT but not the downstream signalling triggered by RAS-RAF effectors.
Conclusions. 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.

PO11
Q3 study: Indicators of quality of cancer care in Southern Switzerland
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Background. Studies on quality of cancer care (QoCC) at the population-based level have been implemented in some regions in Europe, but they are still scarce. A prospective descriptive population-based study focused on three major oncologic pathologies will be conducted in Canton Ticino in a 3-year time period.