Abstracts der Schweizerischen Gesellschaft für Pathologie 2011

77. Annual Meeting of the Swiss Society of Pathology
Luzern, 10.–12. November 2011
PO4 Intratumoral heterogeneity of KRAS and BRAF mutations in primary colorectal cancer and corresponding metastases

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Background. Mutation analysis of KRAS is recommended for metastatic colorectal cancer to identify patients suitable for anti-EGFR based therapies. Here, we test the hypothesis that tissue sampling plays a role in the incorrect assignment of wild-type and mutated gene status in metastatic colorectal cancer patients.

Methods. Mutation analysis for KRAS (codons 12 and 13) and BRAF (codon V600E) was performed using dideoxy sequencing on 48 primary colorectal cancers and 32 matched metastases. Tumor from the most representative block was sampled 5 times each. In total 804 tissue spots were evaluable for KRAS and BRAF.

Results. In primary and metastatic tumors, KRAS mutation occurred in 37.5% and 33.1%, respectively. Frequency of intratumoral heterogeneity was 12.5% at both sites and percent-concordance was 78.8% with kappa value of k=0.56 (95%CI: 0.25-0.87) indicating only moderate agreement. BRAF mutation occurred in 8.3% primary and 6.3% metastatic tumors. Heterogeneity was observed in 3 primary tumors (6.3%) only. All 804 spots were mutually exclusive for mutation in KRAS and BRAF.

Conclusion. Although heterogeneity for KRAS and BRAF occurs in a small number of samples, it may nonetheless lead to incorrect assignment of wild-type or mutated gene status in more than 10% of tumors. This finding could have important clinical implications for patients considered for anti-EGFR-based therapies.

PO5 Different KRAS mutations among populations: possible clinical consequences

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Background. Observational studies of patients with primary colorectal cancer (CRC) have identified KRAS mutation as an important molecular marker. We investigated more directly if there are differences due to number and type of mutations between Ecuadorian patients and Swiss patients.

Methods. Tumor microdissection was performed in all cases. KRAS mutation status was assessed by PCR and direct sequencing in a series of primary CRC specimens collected from 61 Ecuadorian patients from the Instituto Del Cancer Solca in Cuenc, and in a series of 127 Swiss patients from the Institute of Pathology in Locarno.

Results. Of 61 malignant lesions of the Ecuadorian patients 17 exhibited KRAS mutation (27.9% of cases). In particular, we found G13D mutation in 11 cases, G13D in 5 cases and G12A in 1 case (corresponding to 65%, 30% and 3% of KRAS mutated cases). Any G13V mutation was found. Among Swiss patients, 28 (22%) presented a KRAS mutation of which the highest mutation type was the G13D (39%), and in descending order the G13V (33%), G13D (14.20%) and the G12A (1.60%).

Conclusions. Our results outline similarities but also important differences existing among populations. Due to the role of KRAS as prognostic and predictive marker in CRC, these findings could play a relevant role in clinical practice.

PO6 Comparison of different methods for KRAS testing: results of an interlaboratory study of the European Society of Pathology

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Background. Assessment of the reliability and limitations of the most commonly used methods for KRAS testing with special emphasis on samples with low tumour cell content.

Methods. 60 DNA samples and 40 artificial tumour slides with known percentage of KRAS mutated cells (0, 2.5, 5, 10, 15% tumour cells) were distributed to 10 laboratories throughout Europe and analysed with the following test systems each represented twice: Sanger sequencing, pyrosequencing, high resolution melting (HRM), ARMS-PCR with light cycler or ABI read out. Lucerne used a "cold" PCR protocol followed by Sanger sequencing.

Results. Rate of correct results was related to method and lab performance. ARMS-PCR and HRM had the highest concordance rate between the labs and the highest mutation detection rate, but were also the only methods that produced false positive results. In samples with 15% and 10% tumour cell content 9 of 10 labs detected all mutations in the DNA samples and 7 of 10 labs in the tumour slides. The Lucerne protocol was able to detect all mutations in DNA samples with as little as 2% mutated cells and tumour slides with 3% tumour cells.

Conclusions. Every effort should be done to reach a tumour cell content of >20–30%. ARMS-PCR and HRM may rarely produce false positive results. Sanger sequencing combined with "cold" PCR is able to detect most mutations even in samples with very little tumour cells. Since reliability of all methods is laboratory dependent, test validation and participation in external quality assurance programs is mandatory.

PO7 Characterization of MYC and MNT in colorectal cancer patients

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Background. c-Myc oncoprotein is deregulated in several human tumors and its control on cell proliferation is tuned by a series of repressors, whose the most relevant is MNT. In colorectal cancer (CRC), Myc is altered by gene amplification in about 25% cases and MNT loss of heterozygosity (LOH) has been associated with CRC carcinogenesis. As the precise interplay between Myc and MNT has not been fully investigated in tumor specimens, our aim was to better characterize the roles of Myc and MNT in CRC.

Methods. We analyzed 53 sporadic CRC patients. c-Myc gene status was evaluated by FISH, MNT was assessed for LOH using 5 microsatellite loci located inside or near the gene.

Results. We observed c-Myc amplification in 14/45 (31%) evaluable cases. MNT loss was found in 28/45 (58%) informative cases. Out of 39 cases evaluable for both c-Myc and MNT, c-Myc amplification was found in 12/39 (31%) cases and MNT loss in 24/39 (64%) cases. By comparing c-Myc and MNT gene statuses, we observed that c-Myc amplification occurred in 10/35 (40%) tumors with loss of MNT and only in 2/14 (4.28%) cases without MNT loss (p<0.39).

Conclusions. c-Myc and MNT are deregulated in a considerable number of CRC specimens confirming a key role of these genes in CRC pathogenesis. The majority of c-Myc amplified tumors showed MNT loss, possibly implying that a synergistic effect of these alterations is required in CRC development.