705 Genetic alterations of the LKB1 gene in head and neck cancer

S. Ekizoglu[1], E. Kenanli[1], E. Karaman[2], O. Enver[2], T. Ulutin[1], N. Buyru[1]. [1]*Cerrahpasa Medical Faculty, Department of Medical Biology, Istanbul, Turkey,* [2]*Cerrahpasa Medical Faculty, Department of Otorhinolaryngology, Istanbul, Turkey*

Background: Head and neck cancer (HNC) is the sixth most common type of cancer worldwide. The LKB1 gene [also known as STK11 (serine-threonine kinase] is one of the genes which participate in HNC development.
The LKB1 gene is localized on chromosome 19p13.3 spanning 23 kb and is composed of 10 exons, nine of which are coding. The product of the LKB1 gene is a 436-amino-acid protein with a kinase domain (residues 50–319) and a putative carboxyl-terminal regulatory domain with a CCAA-box, a consensus sequence for prenylation. Two nuclear localization signals are located between amino acids 38−43 and 81−84. LKB1 is found both in the cytoplasm and the nucleus. Its intracellular distribution depends on its interaction with binding partners. LKB1 forms a complex with the pseodokinase STRAD and the scaffolding protein MO25 in cells. This interaction regulates its stability, kinase activity and subcellular localization. The LKB1 protein functions as a tumour suppressor that regulates cell polarity, differentiation, and metastasis as well as responses to the energy status to regulate the cell metabolism. Investigators reported that LKB1 is mutated and/or epigenetically inactivated in some sporadic tumours. Therefore, patients with sporadic cancers have also been screened for mutations and approximately 24 different somatic mutations were identified in sporadic cancers. Reported frequencies of the LKB1 gene were 4% (12), 6% (12), 1% (13), 0% (10) and 4% (10) for pancreatic cancer, billiary cancer, hepatocellular carcinoma, colon cancer and testicular tumours, respectively.
Material and Methods: In this study, we analyzed nine exons of the LKB1 gene in tumour tissue and adjacent non-cancerous tissue samples of 50 patients with HNC by direct sequencing.
Results: We detected a novel missense mutation in the nuclear localization signal coding region of the LKB1 gene in 10 tumour samples from patients with HNC. Six different single nucleotide substitutions were also observed in the introns. In two patients a 7 bp duplication was detected in intron 3.
Conclusions: These results indicate that the LKB1 gene may play a role in the etiology of head and neck carcinogenesis.

706 The transcriptional regulator CTCF is involved in the control of ribosomal DNA

D. Delgado[1], M. Rosa-Garrido[1], A. Batlle[1], V. Torrano[1]. [1]*University of Cantabria, Molecular Biology, Santander, Spain*

Background: CTCF is a ubiquitously expressed transcriptional regulator that binds at multiple sites throughout the genome. CTCF is a putative oncosuppressor with several functions such as regulation of cell proliferation, differentiation, apoptosis, enhancer-blocking activity and control of imprinted genes. Our previous studies on CTCF subnuclear localization and in situ run-on assays revealed that CTCF was associated with several components of the nucleolus and influenced nucleolar transcription (Torrano et al JCS 2006). In this work we asked whether CTCF could have a direct role in the regulation of ribosomal genes transcription.
Material and Methods: CTCF binding sites in the human rDNA were searched with a CTCF Binding Sites Data Base (Bao et al NAR 2008). In vitro binding of CTCF and UBF to rDNA was studied by Electrophoretic Mobility Shift Assays (EMSA). Occupancy of CTCF, UBF and modified histones to ribosomal DNA was analyzed by Chromatin Immunoprecipitation (ChIP).
Results: We found several putative CTCF binding sites to human rDNA. EMSA analysis revealed CTCF binding to two sites mapped in the intergenic spacer region 5′ upstream of the ribosomal gene promoter. CTCF binding to both sites was methylation-sensitive. ChIP analysis showed in vivo occupancy of CTCF, as well as its paralogue CTCFL (BORIS), to the rDNA. The transcription factor of the RNA polymerase I UBF plays a critical role on the regulation of ribosomal genes transcription. By serial-ChIP assays we found interaction of both CTCF and UBF at both rDNA sites. Variant histone H2A.Z and marks of active chromatin were present at CTCF binding sites on ribosomal genes.
Conclusions: We have identified two CTCF binding sites upstream of the human rDNA promoter. CTCF in vitro binding and in vivo occupancy at both rDNA sites as well as interaction of CTCF and UBF at rDNA was demonstrated. Our results showed a novel function of CTCF on the ribosomal biogenesis regulation, a critical process linked to the control of cell proliferation and differentiation.

707 Methylation Specific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA): an efficient assay for hMLH1 methylation detection in colorectal cancer

S. Theoleyre[1], M. Blayau[1], C. Dugast[1], N. Rioux-Leclercq[1], M. Denis[2]. [1]*Rennes University Hospital, Rennes University Hospital, Rennes, France,* [2]*Nantes University Hospital, Nantes University Hospital, Rennes, France*

Background: *hMLH1* promoter hypermethylation, in association with *BRAF* mutation, has been shown to be a useful parameter to make the difference, among the colorectal cancers with microsatellite instability (MSI), between sporadic and hereditary non-polyposis colorectal cancer (HNPCC). Therefore, a rapid, sensitive assay for *hMLH1* methylation detection using routine pathological specimens is demanded in clinical practice.
Methods: DNA was extracted from paraffin-embedded sections of colorectal tumours using an automated Bionobis 12GC system. MSI status was determined using a panel of 5 markers (BAT25, BAT26, NR21, NR22, NR24) and samples were considered as MSI colorectal cancers when at least 3 markers were unstable. *hMLH1* methylation was analyzed using the SALSA MS-MLPA kit ME011 MMR (MRC-Holland). This kit includes 5 *hMLH1* specific probes containing a digestion site for the methylation-sensitive HhaI enzyme. Experiments were performed according to the manufacturer's instructions with 100 ng of DNA. Amplification products were separated by capillary gel electrophoresis and then analyzed using the GeneMarker software. Samples were considered hypermethylated when at least 3 probes were methylated. We also evaluated the presence of the *BRAF* V600E mutation by real-time allele-specific PCR and the expression of hMLH1 by immunochemistry (purified anti-human MLH-1, clone G168–728, BD Biosciences).
Results: The sensitivity of MS-MLPA was determined using serial dilutions of enzymatically methylated DNA. This assay allowed us to detect *hMLH1* methylation in samples containing as low as 5% of methylated DNA. We first analyzed 15 microsatellite stable (MSS) tumours. None of them presented a methylated *hMLH1* gene promoter. We then tested DNA extracted from 18 MSI colorectal cancers. Eight of these 18 (44.4%) samples showed positive hMLH1 immunostaining as determined by MS-MLPA. They were all *BRAF* wild type and presented unmethylated *hMLH1*. Among the 10 tumours negative for hMLH1 immunostaining, 8 (80%) presented a hypermethylation of *hMLH1* associated with a *BRAF* mutation in 4 cases (50%). These *hMLH1* methylated tumours most likely correspond to sporadic tumours. The whole procedure can be performed within 48 h (from paraffin-embedded sections to results), and the cost of reagents is only ~20€/test.
Conclusion: MS-MLPA assay offer a high sensitive, robust and accessible approach to the rapid identification of *hMLH1* methylation in MSI colorectal cancer which could be important for the distinction between HNPCC and sporadic colorectal cancers.

708 Regulation of CDX2 expression by the microenvironment in the gastric context

B. Pereira[1], L. David[1], R. Almeida[1]. [1]*IPATIMUP – Institute of Molecular Pathol, Carcinogenesis, Porto, Portugal*

Background: The homeobox transcription factor CDX2 is normally expressed in the adult intestine where it controls the expression of several intestinal genes. However, in certain pathological conditions it is also abnormally expressed in ectopic locations, such as in the stomach, triggering a phenotypic shift known as intestinal metaplasia (IM). Our aim is to study the different cellular and molecular mechanisms leading to *de novo* expression of CDX2 that ultimately dictate this preneoplastic lesion.
Material and Methods: To better mimic the *in vivo* microenvironment in which IM arises, we have established an *in vitro* three-dimensional (3D) cell culture model combining a microporous membrane, a basement membrane matrix (Matrigel®) and human gastric carcinoma cell lines, AGS and MKN45. Expression levels of CDX2 as well as of gastric and intestinal differentiation markers, namely MUC5AC and MUC2 respectively, were assessed in several experimental conditions by real-time PCR, western blot and immunocytochemistry.
Results: The cell lines used show different basal levels of CDX2 protein expression in standard two-dimensional cultures, with detectable expression in AGS and barely detectable expression in MKN45. Most interestingly, after 14 days in 3D culture a decreased expression was observed in AGS, while an increased expression was observed in MKN45, accompanied by a concomitant change in the expression levels of MUC5AC and MUC2. The changes in CDX2 protein levels weren't dependent on transcriptional regulation, since mRNA levels weren't significantly altered, especially in the case of MKN45. A time-course treatment with the protein translation inhibitor cycloheximide revealed that the observed effect in MKN45 was not related with CDX2 protein stability, but rather with new protein synthesis. We then assessed if the observed effect in AGS was related to microRNA mediated inhibition of protein translation. Preliminary results obtained after selectively silencing Dicer expression using RNA interference in the 3D culture showed an increase in CDX2 expression levels, indicating that CDX2 might be regulated either directly or indirectly by specific microRNAs.
Conclusions: The monotypic 3D culture system described highlights the importance of post-transcriptional and post-translational regulation of CDX2, providing insights into possible new regulatory mechanisms that may be determinant for induction and maintenance of the gastric IM phenotype.