Results: To date:
- 14 patients have completed treatment in a frame, 6 in a shell (2 more now on treatment)
- 494 fractions delivered, 478 (97%) are adequate for inclusion, corresponding to 3136 kV images.

Data is available from 158 fractions in shell patients.
- Post correction pre-treatment, translational errors are ⩾2 mm in 0/158 fractions
- Post treatment, errors are ⩾2 mm in 9/158 fractions.

Table: Systematic & Random errors

	Systematic errors						Random errors					
	Translational (mm)			Rotational (°)			Translational (mm)			Rotational (°)		
	Vert	Long	Lat	Yaw	Roll	Pitch	Vert	Long	Lat	Yaw	Roll	Pitch
Shell												
Pre-correction	0.23	1.71	1.30	1.53	0.38	1.08	0.85	1.05	0.72	0.63	0.85	0.90
Post-correction	0.08	0.25	0.21	0.28	0.13	0.16	0.21	0.21	0.26	0.28	0.21	0.24
Post-treatment	0.29	0.39	0.28	0.23	0.23	0.34	0.43	0.45	0.35	0.43	0.27	0.32
Post floor twist (1st 5 fractions)	0.45	0.47	0.35	0.37	0.16	0.45	0.38	0.23	0.28	0.34	0.22	0.22
Frame												
Pre-correction	0.42	0.60	0.30	0.24	0.34	0.37	0.40	0.54	0.42	0.29	0.21	0.22
Post-correction	0.07	0.13	0.07	0.08	0.05	0.09	0.22	0.18	0.17	0.16	0.11	0.20
Post-treatment	0.19	0.26	0.24	0.16	0.09	0.16	0.42	0.29	0.35	0.19	0.16	0.24
Post floor twist (1st 5 fractions)	0.17	0.22	0.25	0.14	0.11	0.19	0.31	0.25	0.20	0.19	0.14	0.19

Conclusion: Data is awaited from the final 2 patients but margin reduction is likely to be safe in shell-immobilised patients when using ExacTrac for daily online correction.

Poster Presentations (Sun, 25 Sep, 09:30–12:00)
Head and Neck Cancer

8520 POSTER

Podoplanin Regulates the Proliferation of Oral Squamous Cell Carcinoma Cells via Its Binding to Extracellular Matrix

M. Tsuneki[1], S. Maruyama[2], M. Yamazaki[1], J. Cheng[1], T. Saku[1]. [1]*Niigata University Graduate School of Medical and Dental Sciences, Division of Oral Pathology Department of Tissue Regeneration and Reconstruction, Niigata, Japan;* [2]*Niigata University Hospital, Oral Pathology Section Department of Surgical Pathology, Niigata, Japan*

Background: We have already reported that podoplanin (PDPN), one of the representative immunohistochemical markers for lymphatic endothelial cells, is enhancedly expressed in carcinoma in-situ and squamous cell carcinoma (SCC) of the oral mucosa, though its pathophysiological function remains largely unknown. Our aim was to determine its molecular role in oral SCC cells, based on a hypothesis that PDPN plays an important role in SCC cell activities such as cell proliferation and migration.
Material and Methods: Surgical specimens of oral SCC were used for immunohistochemistry for PDPN and Ki-67. ZK-1 and the other two SCC cell systems as well as three ACC cell systems of salivary adenoid cystic carcinoma origin were used for in-vitro experiments. PDPN expression levels were determined by quantitative real-time PCR, western blotting, and immunofluorescence. Transient PDPN knockdown was performed with siRNA. Cell growth curves were compared between ZK-1 cells with or without PDPN knockdown or in the absence or presence of anti-PDPN monoclonal antibodies capable of selectively recognizing its cell surface domain. The apoptotic effect of PDPN knockdown in ZK-1 was analyzed by flow cytometry. Their cell attachment, migration, and invasion assays were also conducted by conventional methods using microplates, scratch wounding, transwells, and matrigel.
Results: Immunohistochemically, PDPN was characteristically localized in the peripheral cells of SCC foci where Ki-67 positive cells were simultaneously localized. PDPN was specifically expressed in SCC cells, especially in ZK-1, but not in ACC cells at mRNA as well as protein levels. The cell growth was significantly suppressed in PDPN-knockdowned ZK-1 cells, which was not always due to apoptosis (less than 5%) but rather due to the inhibition of cellular attachment in initial stages after plating in plastic dishes. ZK-1 cell growth was also significantly repressed in the anti-PDPN antibodies in the culture medium. In contrast, migration and invasion activities were not affected at all in ZK-1 cells knockdowned by siRNA for PDPN.
Conclusion: The findings indicated that PDPN functions in cell proliferation but not in migration or invasion of oral squamous cell carcinoma cells by binding with extracellular matrix molecules via its extracellular domain.

8521 POSTER

Novel Candidate Genetic Polymorphisms Identified in Genome-wide Association Study for Base of Tongue Squamous Cell Carcinoma Susceptibility

G.J. Lourenco[1], B. Carvalho[2], R. Pellegrino[3], L. Khater[1], C.T. Chone[4], F.F. Costa[1], C.S.P. Lima[1]. [1]*State University of Campinas, Department of Internal Medicine of the Faculty of Medical Sciences, Campinas, Brazil;* [2]*University of Cambridge, Computational Biology Group of the Department of Oncology, Cambridge, United Kingdom;* [3]*Federal University of São Paulo, Department of Psychobiology, São Paulo, Brazil;* [4]*State University of Campinas, Department of Otolaryngology of the Faculty of Medical Sciences, Campinas, Brazil*

Background: In head and neck, we have traditionally combined squamous cell carcinomas (SCC) of the oral cavity, oropharynx, larynx and hypopharynx in clinical treatment. However, with the advances in our understanding of molecular biology of cancer, the tumours that where once considered to be relatively homogenous diseases are now being recognized as comprising distinct subtypes. Inherited genetic alterations, such as single nucleotide polymorphisms (SNPs), were described in association with oropharyngeal cancer risk in only few reports. Base of tongue (BT) SCC are common tumours of oropharynx; however, the association of SNPs and BTSCC risk is still not clarified and, therefore, this was the aim of the present study.
Methods: DNA of 49 BTSCC patients and 49 controls was extracted using the Qiagen QIamp kit (Qiagen®). Each sample was genotyped individually using DNA high-resolution microarrays containing 500.568 SNPs (SNP array 5.0, Affymetrix®). Further sample processing, including digestion, adaptor ligation, amplification, fragmentation, labelling, hybridization, washing and scanning was assayed according to the standard protocol. Genotype data were acquired by genotyping calling of samples using the corrected robust linear model with maximum likelihood classification algorithm provided by Bioconductor software, as per the recommended guidelines. The differences between groups were analysed by the logistic regression model.
Results: We observed 6.609 SNPs with distinct frequencies between BTSCC patients and controls. 52 SNPs (0.8%) were located in coding sequence (CDS) of amino acids, 51 (0.8%) in 3′ and 5′ untranslated regions (UTR), 3.461 (52.4%) in up or downstream regions and 3.045 (46.0%) in introns. The SNPs were clustered to their main function, evidencing those localized in CDS, 3′- and 5′-UTR, related to cell cycle (*CHFR*, *COSMC*, *ERP29*, *IQCE*, *IRS2*, *KANK4*, *MAU2* and *USP2*), apoptosis (*ARHGEF18*, *CTSB*, *GFRAL*, *JMJD6*, *PIHID1*, *RAB6C* and *SPATA4*), DNA repair (*CCDC6* and *GEN1*), transcriptional process (*GCN2*, *MLL3*, *RPAD1*, and *ZNF415*), cell adhesion (*COL6A3*, *COL22A1*, *IKAP* and *KIND1*) and metastasis (*GCNT1*, *LARS* and *SYNJ2*).
Conclusions: Our preliminary results suggest that SNPs in genes involved in tumour origin and development may predispose individuals to BTSCC. However, these results should be confirmed by functional studies of coded proteins and validated in larger epidemiological studies.
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8522 POSTER

Human Papilloma Virus in Head and Neck Squamous Cell Cancer

I. Asvadi Kermani[1], S. Seifi[1], R. Dolatkhah[1], A. Asvadi Kermani[1], E. Sakhinia[2], A. Ebrahimi[2], A. Lotfi[3], S. Hajalipour[3], S. Dastgiri[4], H.A. Esmaieli[5]. [1]*Tabriz University of Medical Sciences, Haematology and Oncology Research Centre, Tabriz, Iran;* [2]*Tabriz University of Medical Sciences, Biochemistry Department, Tabriz, Iran;* [3]*Tabriz University of Medical Sciences, ENT Department, Tabriz, Iran;* [4]*Tabriz University of Medical Sciences, Social Medicine Department, Tabriz, Iran;* [5]*Tabriz University of Medical Sciences, Pathology Department, Tabriz, Iran*

Background: Epidemiologic and molecular evidence have established the strong link between high risk types of human Papilloma virus (HPV) and a subgroup of head and neck squamous cell carcinoma (HNSCC). We evaluated the frequency of HPV positivity in HNSCC and its relationship to demographic and some risk factor variables in an open case-control study.
Materials and Methods: Fourteen newly diagnosed patients of squamous cell cancer of oropharynx, hypopharynx and larynx aged between 18–50 years were examined from 2008–2010 in Tabriz/Iran. HPV DNA was extracted from paraffin-embedded block of each patient for evaluating by PCR. Saliva samples of 94 control cancer-free subjects were collected for DNA analysis. Multivariable logistic regression method was used to calculate odds ratio for case-control comparisons.
Results: High risk HPV was detected in 6 (42.8%) of patients and 6 (5.3%) of control subjects, which statistically was significant ($p < 0.0001$). HPV-18 was the most frequent type both in the case and control group. HPV-16

DNA was detected in two patients but none was found in control subjects. The relation between demographic and risk factor variables was not statistically significant.
Conclusions: HPV infection has significant impact on HNSCC. Despite HPV-16 possesses the stronger impotent, HPV-18 is more probable to cause malignant degeneration in such cancers amongst some communities. It is a necessity to introduce and conducting immunization program in health care system in order to some extent safeguards such communities.

8523 POSTER

Up-regulation of Neutrophil Gelatinase-associated Lipocalin in Oral Squamous Cell Carcinoma − Relation to Cell Differentiation

K. Noguchi[1], T. Hiromoto[1], M. Yamamura[1], Y. Zushi[1], E. Segawa[1], K. Takaoka[1], K. Moridera[1], H. Kishimoto[1], M. Urade[1]. *[1]Hyogo College of Medicine, Department of Oral and Maxillofacial Surgery, Nishinomiya Hyogo, Japan*

Background: Neutrophil gelatinase-associated lipocalin (NGAL; also known as Lipocalin2, LCN2) is a secreted glycoprotein and elevated expression has been observed in solid tumours. The expression and function of oral cancer, however, is unclear. We investigated the expression of NGAL in oral cancer tissues and oral cancer cell lines.
Material and Methods: Surgical specimens were obtained from 40 patients, including 5 cases of normal mucosa, 5 cases of leukoplakia of the tongue, and 30 cases of tongue cancer (tumour tissue). Tumour tissue were stained H-E for histological examination. Immunohistochemical examination were performed by the ABC staining methods. Moreover, Eight oral carcinoma cell lines (SCCKN, HSC-2, HSC-3, OSC-19, OSC-20, HOC-313, SCC-25, TSU) were lysed in lysis buffer and were performed for Western blot analysis. Gelatin zymography, that were detected for MMP-2 and MMP-9, were performed for condition medium of each cell line.
Results: By immunohistochemical examinations, NGAL expression was strongly up-regulated in well-differentiated OSCC tissues and moderately to weakly in moderately to poorly differentiated OSCC tissues. In contrast, NGAL expression was weak or very weak in the normal mucosa and leukoplakia. By Western blot analysis, NGAL expression levels positively correlated with cell morphology pattern and loss of E-cadherin. In addition, the enzymatic activity of the NGAL/MMP-9 complex significantly correlation with the results obtained by zymographic analysis.
Conclusion: NGAL is highly expressed in well-differentiated cancer, suggesting that NGAL may be a possible role as diagnostic marker of tumour-cell differentiation.

8524 POSTER

A Functional Analysis of Zyxin in Epithelial-mesenchymal Transition of Oral Squamous Cell Carcinoma

M. Yamamura[1], K. Noguchi[1], E. Segawa[1], Y. Zushi[1], K. Takaoka[1], H. Kishimoto[1], M. Urade[1]. *[1]Hyogo College of Medicine, Oral and Maxillofacial Surgery, Nishinomiya, Japan*

Background: Epithelial-mesenchymal transition (EMT) confers destabilization of cell-cell adhesion, cytomorphologic change and cell motility required for cancer invasion and metastasis. Zyxin, one of the focal adhesion-associated LIM domain proteins, is essential for actin reorganization for cell migration. The involvement of Zyxin in EMT was investigated.
Material and Methods: According to the classification of mode of invasion in squamous cell carcinoma of the oral cavity (OSCC) by Yamamoto and Kohama (1984), eight OSCC cell lines were divided: SCCKN, HSC-2, OSC-20 in grade 3, HSC-3, SCC-25, OSC-19 in grade 4C (cord-like type) and HOC-313, TSU in grade 4D (diffuse type). Expressions of E-cadherin, N-cadherin and Zyxin as EMT marker were examined by histocytochemistry and western blot. Cell growth was examined by MTT assay and cell motility was examined by scratch assay and invasion assay. Expression of Zyxin was knocked down using siRNA.
Results: Decreased expression of E-cadherin and increased expression of N-cadherin and Zyxin were found in parallel with mode of invasion from grade 3 to grade 4. Zyxin was markedly expressed in HOC313 and TSU. Treatment of HOC-313 with si-Zyxin resulted in cell morphologic change from spindle to polygonal shape. The si-Zyxin treatment of HOC313 inhibited cell growth and invasion significantly as compared to si-control treatment. When Rho family proteins such as RhoA, Rac1 and Cdc42 were examined, there was no significant difference in expression of RhoA and Cdc42, but expression of Rac1 was weaker in si-Zyxin treatment than in si-control treatment. In addition, expression of Zyxin in HOC-313 was inhibited by Rac-1 inhibitor.
Conclusions: These results indicated that Zyxin may become a possible EMT marker and overexpression of zyxin promotes cell growth and invasion of HOC-313 cells via up-regulation Rac-1.

8525 POSTER

The Prevalence of Microsatellite Instability and Loss of Heterozygosity in Bulgarian Patients With Laryngeal Carcinoma

M. Stefanova[1], S. Jordanov[2], R. Tzveova[1], G. Stancheva[3], A. Mitkova[3], I. Chalakov[2], R. Kaneva[3], V. Mitev[3], T. Goranova[1]. *[1]Medical University − Sofia, Molecular Medicine Centre, Sofia, Bulgaria; [2]UMHAT "Tsaritsa Yoanna − ISUL", Department of ENT/ Clinic of ENT, Sofia, Bulgaria; [3]Medical University − Sofia, Molecular Medicine Centre/ Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria*

Background: A significant proportion of tumours develop through the microsatellite instability (MSI) pathway as a result of impaired DNA mismatch repair. However, data about the prevalence of MSI in head and neck carcinomas are still controversial − some authors have found MSI in more than 40% of the cases, while others claimed that not MSI but loss of heterozygosity (LOH) is often observed in these cancers. In a previous study, high level of epigenetic silencing of *MLH1* gene was found in laryngeal tumours of Bulgarian patients, suggesting involvement of MSI in their development. Thus, we aimed to examine MSI at five loci in patients with carcinoma of the larynx.
Materials and Methods: Forty-eight patients with laryngeal carcinoma were included in the present study. It was approved by the ethical committee of Medical University − Sofia, and informed consent was obtained from all patients. DNA was extracted from fresh frozen tumour tissues and blood samples of the selected patients with laryngeal cancer. Automatic fragment analysis was performed after PCR amplification of the selected microsatellites: D2S123, D5S346, D18S35, FGA and Bat26.
Results: MSI was observed only in one marker − D18S35, in one patient, who had methylated *MLH1* promoter. While MSI was a rare event in the studied tumours, loss of heterozygosity (LOH) was found to be a common feature − 50% of the carcinomas showed LOH in at least one of the five microsatellites. However, no correlation with *MLH1* methylation status was observed. Statistically significant association was found between LOH and the age of patients − LOH occurred frequently in patients over 60 ($p < 0.05$). Also, LOH was observed mainly in heavy drinkers ($p = 0.001$). No MSI or LOH was detected in the group of non-smokers.
Conclusions: Our results show that not MSI, but LOH is a common feature of laryngeal carcinomas in Bulgarian patients. LOH is associated with the age and the impact of external risk factors like alcohol consumption and tobacco smoking.

8526 POSTER

Fibronectin Induces Matrix Metalloproteinase-9 (MMP-9) in Human Laryngeal Carcinoma Cells by Involving Multiple Signaling Pathways

T. Sen[1], A. Chatterjee[1]. *[1]Chittaranjan National Cancer Institute, Receptor Biology and Tumour Metastasis, Calcutta, India*

Background: Cell adhesion to extracellular matrix initiates intracellular signaling cascade regulated by integrin family of receptors. Evidences show that cultured cells in presence of extracellular matrix adhesion molecule Fibronectin (FN) stimulates secretion of matrixmetalloproteinases (MMPs), facilitating cancer cell invasion. Amongst all MMPs, MMP-9 is often reported to play crucial role in tumour cell growth and metastasis.
The present study aims at examining the effects of FN on MMP-9 in laryngeal carcinoma cell line, HEp-2, and understand the molecular mechanism(s) involved.
Materials and Methods: The methods used were gelatin zymography, western blot, semi quantitative and quantitative real-time RTPCR, ELISA, Immunocytochemistry, siRNA studies, MTT assay and inhibitor studies and EMSA.
Result: The study reports that FN induces the activity, mRNA and protein expression of MMP-9 in HEp-2 cells. This effect is mediated mainly by integrin receptor a5b1, since, the blockade of a5 abrogated FN mediated stimulatory responses. siRNA, inhibitor studies and immunoblots suggested involvement of Focal adhesion kinase (FAK), Phosphatidyl-inositol-3-kinase (PI-3K), Extracellular regulated kinase (ERK) and nuclear factor-kappa-B (NF-kB) in FN-mediated MMP-9 induction. Immunocytochemical analysis demonstrated the nuclear localization of ERK, PI-3K and NF-kB. FN-induced transactivation of MMP-9 gene by enhanced DNA-binding activity of transcription factors NF-kB, Activator protein-1 (AP-1) and Specificity protein-1 (Sp1) to the MMP-9 promoter.
Conclusion: This study suggests that extracellular matrix protein FN induces MMP-9 in HEp-2 cells mainly by involving integrin receptor a5b1 and involves activation of multiple signaling pathways which independently or in "cross-talk" to each other finally leads to the transactivation of the MMP-9 gene.