also taken from the same 63 HNSCC patients, 8 dysplasia patients and 29 controls. Pre-treatment serum levels of the following markers were determined; VEGF-A, VEGF-C, VEGF-D and VEGFR1 using commercially available immunoassays. Using a normal oral stromal fibroblast line and a tumour oral epithelial cell line, we investigated the effect of recombinant $VEGF_{121}$ on cell migration in the Boyden chamber. We investigated the effect of recombinant $VEGF_{121}$ on the same cell lines using Western blotting with phospho-specific antibodies to Akt residues Thr308 and Ser473.

Results: The resultant data indicated that both VEGF-A and VEGF-C expression were significantly elevated in cancer patients compared to dysplastic patients. The mean concentrations of VEGF-A, -C, -D and VEGFR1 were higher in HNSCC in comparison to normal ($p = 0.07$; $p = 0.001$; $p = 0.0001$, $p = 0.001$ respectively). The tumour cells were stimulated to migrate through the pores of the filter by VEGF. Cell migration displayed a dose response effect with maximal stimulation at approximately 10 ng/ml VEGF. An inhibitor of PI3 kinase, LY294002, reduced VEGF stimulated migration to baseline levels. The normal fibroblasts, in comparison, were not stimulated to migrate. Akt is activated in some tumours and is a downstream effector molecule in a number of tyrosine kinase receptor pathways. The oral cancer cells exhibited a linear decrease in Akt phosphorylation at Thr308 with increasing VEGF concentration. In contrast, the normal fibroblasts displayed an increase in Akt phosphorylation at Thr308 with increasing VEGF concentration. Phosphorylation of Akt at Ser473 was increased in both cell lines, the degree of phosphorylation being dependent upon VEGF concentration.

Conclusion: The data we have collected increases the knowledge and understanding of oral cancer progression and its possible underlying molecular mechanisms. VEGF expression is increased in our patients with oral cancer and in vitro, the motility of cancer cells is increased by VEGF and can be blocked by inhibitors of the PI3-kinase pathway. This has important implications to tumour angiogenesis, lymphangiogenesis and metastasis. A study using other PI3 kinase/Akt pathway inhibitors may be transferrable to clinical practice in the future.

8594   POSTER

Gene Copy Number as Predictive Marker for Cetuximab Resistance in Head and Neck Squamous Cell Carcinomas

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Background: Cetuximab is a monoclonal antibody directed against the epidermal growth factor receptor (EGFR). It has proven a sufficient treatment in combination with radiotherapy in head and neck squamous cell carcinoma (HNSCC). However, far from all patients benefit from this therapy and predictive biomarkers of response to cetuximab are therefore required.

Materials and Methods: We evaluated the intrinsic cetuximab sensitivity (ICeS) in 35 HNSCC cell lines (established by Professor Grénman, University of Turku, Finland) by a crystal violet assay, and results were expressed as survival compared to control cells. EGFR expression was measured with an ELISA assay and correlation analysis was performed. Five resistant and five sensitive cell lines were selected for gene copy number analysis on Affymetrix SNP 6.0 chips. Single genes representing amplified regions will be verified by quantitative real time PCR (qPCR).

Results: The mean ICeS was 0.76, and the variation was between 0.16 and 1.4. Cell lines with survival exceeding 0.95 were considered resistant, and survival below 0.5 regarded as sensitive. Interestingly, two cell lines proliferated significantly under cetuximab treatment. Twelve cell lines (34%) were resistant to cetuximab, whereas five (14%) were sensitive. The EGFR expression varied greatly among the cell lines. However, there was no correlation between cetuximab sensitivity (ICeS) and EGFR expression ($r^2 = 0.11$). A total of 51 genes were amplified in resistant cells and not in sensitive cells. They were all distributed on two genomic regions, 11q22 and 5p13−15.

Conclusions: Our results show a great divergence in the cellular response to cetuximab treatment. Since the expression of the receptor itself is not an adequate predictive marker, other factors must be uncovered. Chromosome regions 11q22 and 5p13−15 are amplified in Cetuximab resistant cells. Possible driver genes are being evaluated at present.

8595   POSTER

MicroRNA Signature and Functional Characterization of miR-10b in Oral Cancer

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MicroRNA (miRNA) participates in a variety of biological processes, and dysregulation of miRNA is associated with malignant transformation. In this study, we determined specific profile of miRNA associated with oral cancer. Using miRNA array screening method, 23 miRNA were found considerably differential expressions between 6 oral cancer cell lines and 5 lines of normal oral keratinocytes. In which, 10 miRNAs showed the highest significant difference after independent examination by RT-qPCR. Eight molecules were up-regulated; miR-10b, miR-196a, miR-198b, miR-582−5p, miR-15b, miR-301, miR-148b, and miR-128a; and 2 molecules-miR-503 and miR-31 were down-regulated. The miR-10b was further examined, and its functions were characterized in two oral cancer cell lines. The miR-10b actively promotes cell migration (2.6- to 3.6-fold) and invasion (1.7- to 1.9-fold), but has no effect on cell growth or chemo-/radiosensitivity. Furthermore, plasma miR-10b was considerably elevated (20-fold) post-tumour formation in the xenografted mice, suggesting potential application of this molecule in cancer detection.

In conclusion, we have identified at least 10 miRNAs significantly associated with oral cancer, with low P values and high differential expressions. The miR-10b actively participates in cancer formation through promoting cell migration and invasion. There study provides knowledge base for future clinical application of microRNA in oral cancer.

8596   POSTER

Long Standing Goitres Resulting in Malignancies

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Background: To evaluate malignancy rates in long standing goitres.

Material and Methods: Retrospective study of 73 patients with long standing goiters, more than 5 yrs duration, who underwent surgical procedures in our department.

Results: There were 28 males and 45 females. The symptoms ranged from 5 yrs to 30 yrs (mean 13.05 yrs). Twenty-one patients (29%) had histologically proven carcinoma of the thyroid. Malignancy was found in 9 females and 12 males. There were 9 patients with papillary carcinoma, 5 with follicular carcinoma, 6 anaplastic and one with medullary carcinoma. The mean duration of symptoms in patients with maligancies was 13.09 yrs as compared to 12.1 yrs in patients with benign thyroid disease.

Conclusion: Long standing goitres have a high chance of becoming malignant, especially in males.

8597   POSTER

Triple Tracer Molecular Imaging in Advanced Head and Neck Cancer

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Background: Despite progress in treatment of advanced head and neck cancer, cure rate remains unsatisfactory. Assessment of both morphological and molecular characteristics of the tumour would allow optimizing the treatment in the future.

Aim of the study is an assessment of proliferation, glucose metabolism and hypoxia in inoperable, advanced head and neck tumours before and during radical chemoradiotherapy.

Materials and Methods: Between July 2010 and March 2011, 17 patients were included into the study: 9 oropharynx, 3 oral cavity, 3 larynx 2 hypopharynx. All patients were treated by radical chemoradiotherapy, consisting of 70 Gy in 35 fractions and concurrent cisplatin administration: $100 \, mg/m^2$ on days 1, 22, 43. PET/CT with fluorodeoxyglucose (FDG), fluorotymidyne (FLT) and fluoromisonidazole (FMISO) was performed in week preceding start of the treatment. FLT PET was repeated twice during treatment, after 14 Gy and 28 Gy, FMISO was repeated once, after 36 Gy. Primary tumours were manually delineated on contrast CT scans obtained for radiotherapy treatment planning and then automatically on PET scans, using gradient based method. Volumes delineated and standardized uptake values (SUV) were analyzed, and differences were calculated using Wilcoxon Matched Paired Test.

Results: 70 PET/CT images were analysed. Correlation was found between primary tumour volumes delineated on CT scans and FDG and

FLT volumes delineated on PET scans before treatment (R^2=0.7 and 0.8 respectively) No correlation was found for CT and FMISO volumes. Strong correlation was found between volumes of FDG and FLT before treatment (R^2=0.9). Both FLT and FMISO volumes decreased during treatment. Significant differences were found for FLT volumes before treatment and at the first time point (p = 0.02) and for FMISO volumes (p = 0.04).

Conclusions: Assessment of molecular characteristics of head and neck tumours and their changes during treatment may proof a useful to be a useful tool for treatment individualisation in advanced head and neck cancer.

8598 POSTER
Lessons From Culture and Antibiotic Susceptibility of Oral Cancer Flora and its Impact on Perioperative Antibiotics and Post-operative Wound Infections

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Introduction: Peri-operative antibiotic therapy in oral cancer patients has been a subject matter of lot of research and a consensus is yet to be reached. Aim of the study was to study the primary flora of oral cancer and their antibiotic susceptibility.

Methods: Fifty-nine patients were included in the study. Tumour tissue sample was taken in the operation theatre subjected to aerobic culture and susceptibility after induction of anaesthesia but before cleaning, draping and antibiotic injection. Standard peri-operative antibiotics were then given.

Results: Seventy bacteria were isolated from 56 patients. Two patients had sterile culture. One patient had contaminants grown. Thirty nine of seventy (55.7%) bacteria isolated were Gram negative. Most common bacteria were community acquired Pseudomonas, Klebsiella and E. coli. Only 33 (47.1%) bacteria were sensitive to recommended antibiotic prophylaxis of Amoxicillin & Clavulanic acid and Metronidazole while 66 (94.3%) were sensitive to extended antibiotic coverage of Amoxicillin & Clavulanic acid, Amikacin and Metronidazole used at our center. Among Gram-negative bacteria corresponding rates were 9/39 (23%) and 37/39 (94.9%). The corresponding rates for Gram-positive bacteria were 25/31 (80.6%) and 29/31 (93.5%).

Conclusion: A large number of oral cancer patients have their tumours colonized at the time of surgery by Gram-negative bacteria. Extended antibiotic coverage with the addition of an aminoglycoside can cover these bacteria and lead to decreased wound infections.

8599 POSTER
Dietary Risk Factors of Nasopharyngeal Carcinoma − a Case Control Study of Moroccan Population

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Background: The incidence of nasopharyngeal carcinoma (NPC) is relatively high in Maghreb countries. This cancer is a model of multifactorial oncogenesis, but the role of food as risk factor in ethiopathogenesis of this tumour is not negligible. The aim of this study is to identify the association between risk of NPC and some dietary factors in Morocco.

Methods: It is a case-control study including all new cases of NPC (49 cases) hospitalized in the National Institute of Oncology in Rabat between December 2009 and May 2010. Frequency consummation of foods was compared between cases and controls matched for age, sex and residence district (place of residence). A high frequency consummation of a food was defined as consumption once or more by a week. Some traditional foods in Moroccan cooking like Harissa (hot red pepper), Qadid (mutton dried and salted), Khlii (dried meat, salted, spiced cooked and preserved in a mixture of oil and rendered beef fat) and Smen (rancid butter) were analyzed in this study. A conditional logistic regression was used to identify the association between dietary factors and the risk of NPC.

Results: Cases were more likely to have high frequency consumption of Harissa (Odds ratio (OR): 4.05; 95% Confidence Interval (CI): 1.32–12.4), Smen (OR: 4.81; 95% CI: 1.55–14.92) and Black pepper (OR: 3.53; 95% CI: 1.16–10.71), and less frequency consumption of fruits and vegetables (OR: 0.24; 95% CI: 0.08–0.76). There was no significant association between the risk of NPC and the frequency consumption of Qadid, khlii and cooking with olive oil.

Conclusions: Some of these risk factors (Harissa, Black pepper) weren't found in 3 North Africain studies. This study indicates the involvement of dietary factors, and thus the lifestyle in the development of NPC and

the need of biochemical analysis of food specimens to search for the carcinogenic agents.

8600 POSTER
Head and Neck Mucosal Malignant Melanoma Expressing C-kit Might Benefit From New Treatment Option

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Background: Mucosal malignant melanoma (MMM) of the head and neck is rare, constituting less than 1% of all malignant melanomas. There is no clear gender predisposition. Most patients are elderly, median age in studies is between 56−65 years. The prognosis is poor; 5-year survival is between 15−40% according to available literature. New treatment is badly needed to improve patient outcome.

Purpose: To investigate the expression of c-kit, Ki-67, overall survival and local control rate for patients with head and neck malignant mucosal melanoma in the Uppsala region, Sweden, 1998–2010.

Material and Methods: Retrospective analyses of the patient files were performed. Seventeen patients were found, fifteen with primary MMM, two with recurrences (with earlier diagnoses) between 1998 and 2010. The immunohistochemistry was performed with $3\,\mu m$ sections from the operation material and stained with hematoxylin-eosin for c-kit and Ki-67.

Results: All 17 patients had stage III or IV disease. All had primary surgery, four in combination with postoperative radiotherapy. At recurrences all had surgery and 6 patients had radiotherapy as well.

Both 5- and 10-year overall survival was 39%. All pat achieved local control after 3 months, 8 after 1 year and one after 2 years. Nine recurred in median time 9.3 months from treatment cessation, one after 15 months and one after 18.5 years. Nine patients recurred locally and two both locally and with distant metastases.

Fifteen patients expressed c-kit, seven strongly, five intermediately and three weakly. Two were c-kit negative. Thirteen expressed Ki-67 $\geqslant$30%.

Conclusion: This study confirms the poor prognosis for patients with MMM and the low local control rate in this group, regardless of the treatment combination of surgery and radiotherapy. A clear majority of patients in this study express c-kit and have high proliferation in the tumours and metastases as indicated by the Ki-67 rates. Therefore, targeted therapy aimed at the c-kit might present a new treatment option for this group of patients.

8601 POSTER
Current TNM Staging System in Oral Cancer is Faulty: It Needs Amendment

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Introduction: TNM staging system is the universally accepted system for cancer staging including oral cancer. The basic purpose of staging is to predict prognosis and help in planning the treatment. However, the current TNM system in oral cancer do not truly reflect the burden of disease and so the prognosis & treatment strategy. It over and under stage the T disease and do not give importance to number of involved lymph nodes as in breast or colon cancer. Lymph nodes are most important predictor of survival in oral cancer. We have analyzed our data and presenting the potential deficit and problems of current TNM system.

Methods: It is a retrospective analysis of prospective cancer database of our department from 1st August 2006 to 31st December 2010.

Results: Total 523 patients underwent surgery in this period. The M:F was 2.2:1. The age range from 13 to 89 yrs with mean of 49.8 and median of 50 yrs. The most common sites were buccal, alveolo-buccal and tongue. More than 2/3rd cancer were locally advanced (stage III-IV) by current TNM staging system. With median follow up of 18 months, patients with 4 or more nodes involvement have >80% recurrence rate whereas 1−3 LNs involvement have 28% relapse rate. Patients with ENS showed 38% relapse rate. Involvement of both skin and bone (not staged in conjunction) associated with 37% recurrence rate in comparison to involvement of isolated bone or skin involvement (T4) is associated with around 20% relapse rate. Patients with T4 stage without nodal disease have very low relapse rate (10.7%).

Conclusion: Involvement of 4 or more node, extra nodal spread and combined involvement of skin and bone are poor prognostic factors in oral cancer. They are not placed anywhere in oral cancer TNM staging system. There is need to revise the TNM by including these 3 important factors.