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