apoptotic cells was carried out by confocal laser microscope. Monolayer cell adhesion assay was also performed.
Results: NCAM mRNA and protein expressions were found to decrease in a dose-dependent manner upon treatment with cimetidine for 24 h. The MTT assay showed a significant reduction in the number of viable HSG cells. Confocal laser microscopy showed that HSG cells undergo apoptosis by the treatment of cimetidine. The activation of caspases 3, 7 and 9 was observed in HSG cells after treatment with the cimetidine, thus confirming that the apoptosis was induced by the activated caspases. Apaf-1 activity was also detected in a dose-dependent manner in HSG cells after treatment with the cimetidine. The adhesion of HSG cells to neural cells was inhibited by cimetidine.
Conclusions: These findings suggest that the growth, development and perineural invasion of salivary gland tumor can be blocked by cimetidine administration through inducing the apoptosis.

1071 POSTER

Effects of preoperative chemotherapy on metastasis for oral squamous cell carcinoma in the mice model

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The presence or absence of metastasis bears an important influence on the prognosis of head and neck cancer patients. Recently, neoadjuvant chemotherapy has become widely used as an initial treatment. However, the true effectiveness of neoadjuvant chemotherapy on metastasis is still unestablished. Therefore, using an orthotopic implantation model in which the cervical lymph node metastasis of oral squamous cell carcinoma can be reproduced, we investigated the inhibitory effect of neoadjuvant chomotherapy on metastasis.
Material and Methods: A highly invasive and metastatic human oral squamous cell carcinoma cell line, OSC-19 cells, was implanted into the tongues of nude mice. After implantation, the mice were divided into four groups, Group S (surgery group), Group C+S (preoperative chemotherapy + surgery group), Group S+C (surgery + postoperative chemotherapy group), and a control group (nontreatment group). The treatment (tumor resection or chemotherapy) was started 7 days postimplantation. The effects of each treatment on cervical lymph node metastasis in each group were investigated by examining the rate of lymph node metastasis formation at 28 days postimplantation.
Results: In the control group, five of the 11 mice died of cachexia before the end of the experiment. However, all mice in Group S, Group C+S, and Group S+C survived until 28 days after implantation. The cervical lymph node metastasis rates were 81.8% in Group S, 18.1% in Group C+S, 63.6% in Group S+C, and 100% in the control group. Thus, metastasis to the cervical lymph node was markedly inhibited by the combination of neoadjuvant chemotherapy and tumor resection.
Conclusion: The findings in this study indicate that neoadjuvant chemotherapy is effective for inhibiting metastasis, and that it is necessary to begin chemotherapy as early as possible during the therapy to obtain an inhibitory effect on metastasis. Considering these effects, if anticancer drugs are used better therapeutic results can be expected.

1072 POSTER

Microsatellite alterations in head and neck squamous cell carcinoma: prognostic value and correlation with pimonidazole

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Introduction: As most tumors, HNSCC (Head and Neck Squamous Cell Carcinoma) arises as a consequence of multiple cumulative genetic alterations. This genomic instability is partly induced by the tumoral micro-environment of which hypoxia is a characteristic pathophysiologic parameter. Hypoxia can be measured by extrinsic markers like pimonidazole. Genomic instability is, amongst others, characterized by MSI (microsatellite instability) and LOH (loss of heterozygosity). MSI arises as a consequence of a deficient DNA MMR (mismatch repair) system leading to an accelerated accumulation of nucleotide mutations and changes in length of short, repetitive microsatellite sequences which are spread throughout the entire genome. LOH is another feature of genomic instability, being a mechanism to inactivate tumor suppressor genes. The aim of the project was to find out if a correlation exists between MSI/LOH and pimonidazole and to evaluate the prognostic value of both parameters.
Materials and methods: 57 patients with HNSCC were included so far, of which 27 are already evaluated. For the evaluation of tumoral hypoxia, we used immunohistochemical stainings for pimonidazole on paraffin embedded material coming from the resection specimen. Multiplex polymerase chain reaction (PCR) with 14 fluorescence-labeled forward primers selected from literature was used to assess MSI and LOH. The obtained fragments were analyzed by the ABI PRISM3100 genetic analyzer.
Results: We found LOH in 1 marker in 11.1% of all patients (3/27). LOH in two or more markers was found in 29.6% (8/27). MSI in 1 marker was found in 14.8% of all patients (4/27), while MSI in 2 or more markers was found in 7.4% (2/27). There was a correlation between pimonidazole and N-stage ($p = 0.020$) and disease-stage ($p = 0.047$). LOH was significantly associated with N-stage ($p = 0.034$) and disease-stage ($p = 0.044$). No correlation was found between pimonidazole and outcome. Patients expressing either MSI or LOH had a poorer outcome than patients without these genomic alterations (NS). Finally, patients with either MSI or LOH tended to have higher pimonidazole values (NS).
Conclusion: In this prospective study on genomic instability in 27 patients with HNSCC treated by surgery we could see a trend for a correlation between MSI/LOH and outcome, and MSI/LOH and pimonidazole. We will try to validate these findings in a total of 57 patients. These results will be presented at the time of the meeting.

1073 POSTER

Stromal cell-derived factor-1 (SDF-1) gene polymorphism in patients with head and neck cancer

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Background: SDF-1 is a CXC chemokine with key roles in tumor growth, angiogenesis and metastasis of different types of tumors including osteosarcoma, small cell lung cancer (SCLC), pancreatic, prostate and breast cancers. This chemokine has a G>A mutation at position 801 in 3?-untranslated region, which is present in its β transcript, known as SDF1−3?A. This investigation was aimed to study the frequency of SDF1−3′A in patients with head and neck cancer.
Patients and Methods: Genotype and allele frequencies of 149 patients, 113 (75.8%) males and 39 (24.2%) females, with head and neck cancers and 262 cases of normal healthy individuals were investigated by PCR-RFLP method.
Results: Data indicated that 42 (28.2%) patients and 145 (55.3%) controls had GG genotype, 99 (66.4%) patients and 97 (37%) normal individuals had AG genotype while 8 (5.4%) patients and 20 (7.7%) controls had AA genotype. The comparison of genotypes frequency was statistically significant between patients and controls ($P = 0.00000006$).
Conclusion: SDF-1 and its exclusive receptor CXCR4 interaction seems to play a significant role in biology of tumor cell metastasis and migration. Considering the previous findings on high producer and low producer of SDF-1 alleles, it is concluded that SDF-1 alleles probably has diverse effects on susceptibility of patients with head and neck cancer.

1074 POSTER

Correlation between tissue plasminogen activation system and clinicopathological parameters in thyroid cancer

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The plasminogen activation system plays a crucial role during cancer invasion and metastasis. A large body of clinical data indicates that high levels of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) can be used to predict poor patient prognosis for multiple types of solid tumors. Little is examined about prognostic relevance of plasminogen activation system in thyroid cancer. It was found that uPA, its receptor (uPAR) and its inhibitor (PAI-1) were expressed diffusely in most thyroid cancers. These findings suggest that plasminogen activation system is functionaly active in thyroid carcinoma, but no relationship between the expression of these proteins and clinicopathological parameters could be determined. The purpose of the present study was to investigate the relationship between cytosol concentrations of uPA and PAI-1 in thyroid carcinoma tissues and thyroid carcinoma clinicopathological prognostic factors. Determinations of uPA and PAI-1 concentrations were made using enzyme-linked immunosorbent assays in thyroid tumor and normal tissue cytosol samples of 23 patients (median 56 y., range [3−76], 18 female, 5 male). All patients were classified according to standard clinicopathological parameters. Significantly higher levels ($p < 0.001$) of uPA and PAI-1 were found in thyroid cancer (mean

uPA was 1.11±2.71 ng/mg protein, median uPA 0; range [0.0–10.0], mean PAI-1 15.04±29.45 ng/mg protein, median PAI-1 4.31; range [1.35–136.70]) compared to normal thyroid tissue (mean uPA 0.004±0.147 ng/mg protein, median 0; range [0.000–0.060], mean PAI-1 2.34±0.35 ng/mg protein, median 2.33; range [1.52–3.00]). There was a positive correlation between the levels of uPA and PAI-1 concentrations in cancerous tissue ($p < 0.001$, correlation coefficient 0.72). Both uPA and PAI-1 levels were associated with carcinoma differentiation, larger tumor size and these two proteins exhibited distinct rise if distant metastasis were present. The uPA and PAI-1 levels showed significant difference among various histological type of thyroid cancer. However no significant association between uPA and PAI-1 levels and extrathyroid invasion was found.
These data suggest that significant correlations exist between uPA and its inhibitor PAI-1 and the standard prognostic parameters in thyroid cancer. Further investigations have to clarify whether uPA and PAI-1 could be independent prognostic factors in thyroid cancer.

1075 POSTER

P53 and Ki-67 expression in distinguishing follicular adenoma from follicular carcinoma of thyroid

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Background: Distinguishing between follicular adenoma and follicular carcinoma of the thyroid can be particularly challenging when performing routine diagnostic pathology, and often requires the examination of several histological sections in order to identify the presence of unequivocal capsular and/or vascular invasion.
Material and methods: To investigate the use of immunohistochemical markers in the differential diagnosis of follicular lesions of the thyroid, we studied the expression of P53 and Ki-67 in 52 follicular adenomas and 52 follicular carcinomas of the thyroid. As a control, we examined 30 simple goiters.
Results: Ten percent (10%) of simple goiters exhibited nuclear P53 expression, compared with 55.8% for follicular adenomas, and 82.7% for follicular carcinomas. Nuclear Ki-67 expression was observed in 30% of simple goiters, 51.9% of follicular adenomas and 96.2% of follicular carcinomas. The sensitivity and specificity of using p53 to diagnose follicular carcinoma from follicular adenoma was 82.7% and 44.2% respectively, while for Ki-67 the sensitivity and specificity was 96.2% and 48.1% respectively. When analyzing both markers together the sensitivity and specificity was 82.7% and 57.7% respectively.
Conclusions: The results of this study indicate that immunohistochemical detection of P53 and Ki-67 may have practical utility in the differential diagnosis of follicular carcinomas from follicular adenomas in routine thyroid surgery.

1076 POSTER

Validation of dynamic contrast enhanced MRI parameters as surrogate markers of hypoxia in squamous cell carcinoma of the head and neck

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Background: Hypoxia is an important determinant of response to radiotherapy and correlates with outcome in squamous cell carcinoma of the head and neck (HNSCC).
A non-invasive method for identifying areas of reduced oxygenation within tumours may enable radiotherapy planning and delivery to be individually optimised.
Aim: To validate established parameters derived from dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) as surrogate markers of hypoxia in squamous cell carcinoma of the head and neck (HNSCC).
Methods: 144 measurements of DCE-MRI parameters from 12 regions of interest (ROIs) were made in 4 patients with HNSCC prior to surgical resection. Examinations were performed on a 1.5 T Philips Intera®. An axial 3D scan at the centre of the tumour (TR = 4 ms, TE = 1.23, flip angle=2º) comprising 15.2 mm contiguous slices was followed by a geometrically identical DCE scan (TR = 4 ms, TE = 1.23, flip angle=10º) repeated continuously over 72 6.4 sec time-points. On the 10th repetition 0.1 mmolkg^{-1} of Magnevist®, was administered intravenously (iv) at a rate of 4 ml/sec. Scans were calibrated so intensity changes due to the passage of contrast agent were converted to changes in contrast concentration. Data were processed to produce quantitative contrast concentration curve descriptors (area under the curve, AUC; time to peak, TTP; time to onset, T1onset) and kinetic parameters associated with permeability (Ktrans, Kep) and extra-cellular, extra-vascular space (Ve). 0.5 g/m^2 pimonidazole hydrochloride was administered iv 16–20 hours before surgery. At resection the tumour was orientated such that the pathological specimen was sectioned in the image plane. The pimonidazole uptake was identified by immunohistochemistry. A histological section within the tumour was matched to the corresponding image slice and corresponding ROIs drawn on both the image slice and the section. The percentage of pimonidazole staining within the ROIs defined the hypoxic fraction. Correlations between the DCE-MRI parameters and the hypoxic fraction were assessed using the Spearman rank correlation coefficient (Rs).
Results: See Table 1.

DCE-MRI parameter	Ktrans	Kep	Ve	T1onset	AUC	TTP
Rs	0.753	0.595	−0.38	−0.231	0.697	−0.306
(Rs 95%CI)	0.298 to 0.929	0.013 to 0.876	−0.79 to 0.266	−0.72 to 0.411	0.187 to .911	−0.757 to 0.342
P-value (2-tailed)	0.00	0.05	0.24	0.44	0.01	0.36

Conclusion: These preliminary results suggest that pharmacokinetic parameters derived from DCE-MRI may be surrogate markers of hypoxia in HNSCC. This non-invasive, spatial mapping of intratumoural hypoxia may enable targeted radiation dose escalation to radioresistant clonogens with the potential for improved local control and survival in this group of patients.

1077 POSTER

The significance of stromal desmoplasia and the appearance of myofibroblasts at the invasive front in squamous cell carcinoma of the oral cavity

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Background: The phenomenon of tumor invasion into the extracellular matrix in stroma has gradually been clarified to involve ingenious interactions between tumor and stromal cells. However, the underlying mechanisms of tumor desmoplasia and its biological significance in tumor growth, i.e., as a host defense mechanism or a scaffold for tumor invasion, remain unclear. Myofibroblasts has known to be a major player for fibrosis or constitution in granulation tissue. We examined extent to which the connective tissue in tumor stroma vary due to the histological grade of malignancy, and whether myofibroblasts play a role in assisting cancer invasion and metastasis.
Material and methods: Biopsy materials from 84 patients who had not yet undergone any treatment for oral squamous cell carcinoma (OSCC) and 11 samples of normal oral mucosa were used in this study. The data of semi-quantification analysis for intra-stromal collagen fiber which was performed by color distinguish technique using digital image of Azan staining and that of the immunohistochemical identification of myofibroblasts characterized with smooth muscle actin, vimentin, and desmin at the invasive front, was compared to clinicopathological parameters (mode of cancer invasion: Grade 1–3/4C/4D, degree of differentiation: low/moderate/high, T-categories: T1–4, pathological lymph nodes metastasis: pN+/−).
Results: Image analysis of stromal collagen fiber with regard to the histological mode of cancer invasion showed a unique feature with two peaks of both ends (normal control and grade 4D). In particular, there were few myofibroblasts in the low invasive mode (Grade 1–2), while myofibroblast-positive cases showed a significant increase from a moderate to high invasive mode (Grade 3–4D) ($p < 0.01$). Myofibroblast-positive cases were significantly increased along with the degree of differentiation ($p < 0.05$). Lymph node metastasis was found at a higher incidence among myofibroblast-positive cases than among negative cases ($p < 0.05$).
Conclusions: Fibrous stroma in OSCC showed a desmoplastic response, indicating an invasion protection system and tissue reconstruction as shown in grades 1 to 4C, whereas it is suggested to acquire an independent invasive mechanism in grade 4D that is essentially different from that in other grades, indicating the most frequent failure of loco-regional control, and tumor desmoplasia is one part of the stromal representation forms in highly-developed invasive tumors.