invasion ($P = 0.013$); greater cytoplasmatic expression was revealed with a univariate analysis in the female gender ($P = 0.0025$); with the presence of extra-thyroid extension ($P = 0.037$), perineural invasion ($P = 0.012$) and multicentric tumors ($P = 0.005$); 3) the greatest cytoplasmatic expression was associated to the greater Ki-67 expression and to the 3 compartments with the greater caspase-3 expression. In conclusion, it was shown the possible application of the galectin-3 as a tumor marker in well differentiated thyroid carcinoma and nucleolus expression, as an indicator of its metastasis potential.

1067 POSTER

Angiogenesis as an indicator of metastatic potential in papillary thyroid carcinoma

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Background: Angiogenesis is new blood vessel formation, a process that can lead to tumor development. Microvessel count has been correlated to metastasis in some neoplasias.
Purpose: To determine if measurement of microvessel density is useful in predicting metastasis to the cervical lymph node and prognosis in patients with papillary thyroid carcinoma.
Methods: A retrospective analysis was performed in 30 patients that had undergone total thyroidectomy. They were divided in 2 groups of 15 patients – with and without metastatic disease. Immunohistochemistry was used to detect expression of CD34 in archival paraffin-embedded papillary thyroid tumors, and microvessel density was calculated based on it. Association between microvessel density and the presence of metastasis, according to histological subtype, disease recurrence, and AMES prognostic index groups was determined through statistical analysis.
Results: The median microvessel density for the patient group without metastasis (200.0 microvessels/mm^2) was apparently, but not significantly, less than that observed among metastatic disease patients (254.4 microvessels/mm^2) ($P = 0.20$). When papillary carcinoma subtypes were analyzed, this difference became significant ($P = 0.02$): the follicular variant exhibited a greater microvessel density than the other subtypes, independent of metastasis presence. There was an apparent, but not significant, tendency for a larger median microvessel density in the group of patients that presented recurrence (294.4 microvessels/mm^2 vs 249.6 microvessels/mm^2, $P = 0.11$). There was no relationship between risk level and microvessel density: in the low- and high-risk groups, the median MVD was 304.0 microvessels/mm^2 and 229.6 microvessels/mm^2, respectively ($P = 0.27$).
Conclusions: The results suggest that angiogenesis is more intense among metastatic tumors in the classic and the tall cell variants, indicating that microvessel count can be an indicator of the potential for metastasis in these subtypes of papillary thyroid carcinoma. Patients that developed recurrent disease had a tendency to exhibit higher angiogenesis; however, there was no association between microvessel density and the AMES prognostic index.

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Human papillomavirus types in head and neck cancer: an analysis of patients with unresectable head and neck squamous cell carcinomas (HNSCC) treated with curative radiochemotherapy (RCT)

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Intoduction: Over the past 15 years, human papillomavirus (HPV), the necessary cause of cancer of the cervix, has also been etiologically linked with a subset of HNSCC. The true prevalence of HPV and its influence on clinical outcome remain uncertain. Highly sensitive detection techniques, such as nested polymerase chain reaction (PCR) identified HPV DNA in a substantial proportion (30%-50%) of nonmelanoma skin cancer. Cutaneous HPV types might play a role in head and neck cancer but clinical data are missing.
Purpose: This study was performed to evaluate retrospectively HPV prevalence, genotypes and influence on clinical outcome in a subset of patients with unresectable HNSCC treated with RCT in a multicenter randomized German trial at the University Hospital of Heidelberg, Germany.
Methods: Thirty-six paraffin embedded biopsies of patients treated with curative RCT (total RT-dose 69.9, 600 mg/m^2/dy 5-FU; 70 mg/m^2 carboplatin days 1–5 and 29–33) were analysed with single-phase primers FAP 69/64 and CP 65/70 and a nested PCR of FAP and CP to detect the majority of cutaneous and mucosal HPV types. The experimental findings were correlated with overall survival (OS), progression-free survival (PFS), distant metastases-free survival (DMFS).
Results: Ten out of 36 tumors were positive for high risk cutaneous HPV-types(1 ct HPV 5, 3 HPV 8, 4 ct HPV 9, 1 HPV 20, 2 ct HPV 48, 2 ct HPV 50, 2 HPV 76, HPV 79) Seven biopsies showed multiple HPV-DNA. HPV-infection was independent of tumor site (oropharynx versus hypopharynx). Kaplan-Meier survival estimates showed no difference in OS, PFS and DMFS between patients with HPV positive compared to negative tumors.
Conclusion: The role of cutaneous HPV in oncogenetic processes is not yet clarified, but they might also play a role in HNSCC. In contrary to our previous data which demonstrated HPV 16 positive HNSCC have a better prognosis than HPV negative tumors, there seems to be no evidence of a positive influence on clinical outcome or preferred tumor localization for tumors positive for cutaneous HPV.

1069 POSTER

Loss of heterozygosity on chromosomes 2q21 and 19p13.2 in oxyphilic thyroid cancers

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Hürthle thyroid tumors are characterized by frequent numerical chromosomal aberrations, including aneuploidy or polyploidy, losses and gains of some chromosomal regions and DNA fragmentation. In recent years great attention has been paid to the combined analysis of morphological and genetic features of oxyphilic tumors and to the elucidation of their pathogenesis. We analyzed for loss of heterozygosity (LOH) of the candidate regions for *TCO* (Thyroid Tumor with Cell Oxyphilia) and *NMTC1* (Non-Medullary Thyroid Carcinoma 1), two loci already mapped on chromosomes 19p13.2 and 2q21 respectively. Matched normal and tumor DNA samples from 70 patients with sporadic oxyphilic thyroid tumors and 20 with sporadic follicular tumors were subjected to microsatellite analysis using 10 markers on 19p13.2 and 6 markers on 2q21. This approach led us to the observation of a more significant LOH in oxyphilic than in follicular tumors. Allelic loss in tumor samples was evenly distributed in both 19p13.2 and 2q21 regions, in accordance with the established linkage of *TCO* and *NMTC1* for inherited tumors. In order to investigate the possible contribution of both susceptibility loci in oxyphilic tumors, the family which led to the original mapping of *TCO* locus was reanalyzed for the markers in the 2q21 region. This led to the exclusion of linkage with the *NMTC1* locus and to the refutation of the digenic inheritance hypothesis at least in this family.

1070 POSTER

Cimetidine: a lethal weapon against malignant salivary gland tumor?

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Background: It has been reported that cimetidine inhibits the growth of glandular tumor such as colorectal cancer, however, the effect of cimetidine against salivary gland tumor is still unknown. We demonstrated previously that human salivary gland tumor (HSG) cells spontaneously express neural cell adhesion molecule (NCAM) and that the proliferative activity of HSG cells could be controlled via homophilic binding mechanism. In the present study, we investigated whether cimetidine could prevent the growth of HSG cells.
Materials and methods: The HSG cell line, derived from a human submandibular salivary gland, was established by Shirasuna et al. and three human oral squamous cell carcinoma cell lines, HSC-2, 3 and Ca9–22 were respectively maintained in 25 cm^2 culture flasks at 37ºC in a humidified 5% CO2 incubator until required. Northern-blot, Western-blot analysis and MTT assay were performed. Morphological observation of

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.

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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.

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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.

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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