bronchopulmonary carcinoid tumors in relation to the immnuohistochemical expression of Bcl-2 family proteins Bax and Bcl-2 and correlate it with clinical and histopathological variables. We analyzed Bax and Bcl-2 expression in 44 patients subjected to resection of bronchopulmonary carcinoid tumor, using immunohistochemistry technique. This study involved 43.2% men and 56.8% women with an average age of 38.8 years. Considering the tumor size they presented a mean of 28.3 mm. Bax and Bcl-2 proteins were expressed in 16 (36.4%) and 22 (50.0%) tumors, respectively. Univariate analysis found significance between positive immunostaining for Bax and lymph node metastasis ($P = 0.024$) and death ($P = 0.024$). There was no siginificant relation between Bcl-2 expression and the study's variables. The association between Bax and lymph node metastasis is important in clinical practice, once they may determine the prognosis of bronchopulmonary carcinoid tumor. Further series are required to fully assess the role of Bcl-2 family protein expression in these tumors, reaffirming the relevance of apoptosis studies.

1182 PUBLICATION

Heparanase expression in lung carcinoid tumors by immunohistochemistry

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At present, there is no immunohistochemistry marker that reliably predict which pulmonary carcinoid will behave aggressive. So, we focus our studies to investigate a possible new marker for lung carcinoid tumors and we choose heparanase, an endo-β-glucuronidase, that seems to be involved in tumor development promoting degradation of heparan sulfate proteoglycans. We analyzed heparanase expression in 40 patients subjected to resection of lung cancer comparing with 28 normal lung tissues obtained from non neoplasic area at the same patients. Immunohistochemistry assay was performed using polyclonal antibody HPA2-C17 (Santa Cruz). We analyzed 10 microscopic fields at a magnification of 400x using ImageLab 2000 software. The variables considered were: sex, age, specific localization, histologic criteria, tumor size, presence of metastasis and percentage of positive cells for heparanase expression. Association between variables was assessed by univariate analysis by Students t-Test for parametric variables using the SPSS10 software. This study involved 59% men and 41% women with an average of 39.16 years old; 88% of lung carcinoids represented tipical tumors; however, 12% were atypical. Tumor size mean was 29.55 mm and only 30% of the patients had metastasis. We observed a significant difference between heparanase expression and lung carcinoids comparing with normal tissues ($p < 0.0001$), since 75% of carcinoids tumors were positive for heparanase immunohistochemistry, while 100% of normal tissues were negative. In addition we also obtained a relation between tumor size ($p < 0.0001$) and heparanase expression ($p = 0.022$) in atypical lung carcinoids. This study validated the importance of heparanase expression to explore the presence of lung carcinoid. In addition, we conclude that atypical lung carcinoids presented a higher heparanase expression and bigger tumor size when compared with typical carcinoids which are less aggressive tumor. (Supported by FAPESP, CAPES and NEPAS).

1183 PUBLICATION

Methylenetetrahydrofolate reductase (MTHFR) polymorphisms affect chemotherapy response in lung cancer patients

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A growing body of evidence indicates that folate status during chemotherapy might influence chemoresponse and long-term survival. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme crucial to the folate pathway. This study investigated whether the previously described SNPs *MTHFR C677T* and *MTHFR A1298C* have an influence on chemotherapy response in lung cancer patients. In both SNPs, the variant allele is known to reduce enzyme activity, causing aberrant methylation among other possible effects. For 349 Caucasian patients with primary lung cancer (162 SCLC, 187 NSCLC) that were recruited in the Thoraxklinik Heidelberg from 1999 to 2004, the response to first line chemotherapy after the 2nd cycle was assessed according to the response evaluation criteria in solid tumours (RECIST). DNA was isolated from peripheral blood and genotyped for *MTHFR* polymorphisms by PCR followed by fluorescence based melting curve (LightCycler®) analysis. Genotype frequencies were compared in responders (complete or partial tumour remission) and non-responders (stable or progressive disease). Presence of at least one *MTHFR 1298 C* variant allele was significantly associated with better chemoresponse (OR 0.26, 95% CI 0.11 – 0.61) in SCLC patients who received etoposide, while no correlation was observed for the *MTHFR 677* variant allele in the same group (OR 1.31, CI 0.57 – 3.02). One previous study (Alberola et al., Clin Lung Cancer, 2004) examined the influence of the *MTHFR C677T* polymorphism on chemoresponse in NSCLC patients and found no significant differences. In contrast, we found the presence of at least one *MTHFR 677 T* allele to be significantly predictive of a better outcome in NSCLC patients who received chemotherapy with either cis- or carboplatin (OR 0.44, CI 0.21–0.92), while the *MTHFR A1298C* polymorphism showed no significant correlation in this group.
In conclusion, different variant alleles, both associated with reduced enzyme activity, were found associated with favourable chemotherapy response in the two main groups investigated. Whether this predictive function of the two MTHFR polymorphisms is the result of different mechanisms affecting enzyme activity or is due to differences between the two histological groups of SCLC and NSCLC tumours or due to the different choices of chemotherapeutic drugs remains to be further investigated.
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1184 PUBLICATION

Increased risk for non-small cell lung cancer in carriers of a genetic functional variant in leptin gene

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Background: Leptin is a pleiotrophic hormone with angiogenic and proliferative potential. The long isoform leptin receptor is present in the lung, suggesting a possible peripheral action of this hormone in that organ. Leptin is further associated to the development of several cancer types in organs were it interact with cell receptor.
Material and methods: In this study, 140 patients with histological diagnose of Lung Cancer and 341 healthy controls were genotyped for a leptin gene functional variant (*LEP*–2548 G/A).
Results: In this study, the homozygous AA genotype of the polymorphism in the 5' flanking region of the leptin gene was found to be associated to lung cancer ($P = 0.011$). This overexpressing genotype increased by 2-fold the risk for non-small cell lung cancer ($P = 0.015$). Age-adjusted logistic regression analysis in men indicates an association of AA genotype with all lung cancer cases (OR, 3.23; 95%CI, 1.49–7.02), non-small cell lung cancer type (OR, 3.41; 95%CI, 1.54–7.59), and the histological subtypes squamous cell carcinoma (OR, 3.19; 95%CI, 1.26–8.13) and adenocarcinoma (OR, 4.29; 95%CI, 1.64–11.72). Furthermore, multivariate logistic regression analysis confirmed the AA genotype (OR, 2.57; 95%CI, 1.34–4.92), male gender (OR, 13.16; 95%CI, 6.86–25.24) and age over 63 years (OR, 2.27; 95%CI, 1.41–3.66) as risk factors, demonstrating the independent association of AA genotype and lung cancer development. Kaplan-Meier analysis showed a younger age for lung cancer onset in AA carriers, in comparison to non-AA carriers (log rank test, $P = 0.023$).
Conclusions: This study provides evidence of a role for the genetic *LEP* functional variant in non-small cell lung cancer. Our results suggest an etiopathological role for leptin in lung cancer and support the hypothesis of *LEP* functional polymorphism influence in cancer behaviour.

1185 PUBLICATION

Relationship between expression of xiap protein in operable non-small cell lung carcinomas and apoptosis index and postoperative prognosis

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Background: Dysregulation of apoptosis plays an important role in carcinogenesis, tumor progression, and resistance to chemotherapy. X-linked inhibitor of apoptosis (XIAP) is considered to be the most potent

caspase inhibitor of all known IAP (inhibitor of apoptosis) family members. This study was designed to assess the pattern of expression and the prognostic value of XIAP in radically resected non-small cell lung carcinoma (NSCLC) patients.
Method: The expression of XIAP and its relationship with clinicopathologic parameters (patient age, TNM stage, TNM-pT, TNM-pN, histologic type, VEGF expression, microvessel density, PCNA index) and overall survival were analysed with formalin-fixed, paraffin-embedded blocks from eighty cases of NSCLC. In addition, the apoptotic index (AI) was also assessed.
Results: In a regard to histologic type, squamous cell carcinoma (SCC) showed XIAP expression in 91.3% (42/46) and adenocarcinoma (AC) in 61.8% (21/34). The difference was significant ($p = 0.001$). There was no correlation between XIAP expression and other parameters. In the group of AC, XIAP expression showed the signifcant correlation with older age group $\geqslant$58 years and VEGF expression ($p = 0.028$, $p = 0.014$, respectively). The AI in the group with or without XIAP expression were $2.5 \pm 4.9\%$ and $18.5 \pm 28.9\%$, respectively ($p = 0.001$). Both groups just aforementioned showed no significant difference in median survival time (42.5 months, 29.8 months, respectively).
Conclusion: This study suggests that the XIAP expression in NSCLCs could have relation to inhibition of apoptosis, and show differential expression according to histologic type. However, its prognostic role during the progression of NSCLC needs to be further defined.

1186 PUBLICATION

Expression of MAGE-D4 is correlated with tumor-cell proliferation of non-small cell lung cancer

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Purpose: MAGE-D4, originally named as MAGE-E1, is a novel MAGE family gene that was highly expressed in malignant tumors, but less expressed in normal tissues. in the present study, we examined expression and intracellular localization of MAGE-D4 protein in NSCLC, and revealed the following findings: 1) expression of MAGE-D4 mRNA was up-regulated in tumor tissues compared with the normal lung, 2) intracellular distribution and morphological changes according to cell-cycle of MAGE-D4 was similar to those of the tubulin, 3) enhanced MAGE-D4 expression was correlated with higher proliferative activity.
Experimental Design: Expression of MAGE-D4 protein was estimated by immunohistochemistry. MAGE-D4 mRNA expression was also studied by quantitative reverse transcription–PCR (RT-PCR).
Results: We assessed MAGE-D4 expression in NSCLC tissues. MAGE-D4 expression was up-regulated in tumor tissues compared with normal lung tissues (mean MAGE-D4/GAPDH values, 0.035 for tumor tissues and 0.009 for normal lung tissues; $P = 0.002$), but no significant difference in MAGE-D4 expression according to the pathologic stage. Proliferative activity of tumor cells was significantly higher in high MAGE-D4 tumor (mean proliferative indices, 58.3 for high MAGE-D4 tumor and 34.0 for low MAGE-D4 tumor; $P < 0.001$). In addition, high MAGE-D4 expression was more frequently seen in squamous cell carcinoma than in adenocarcinoma ($P = 0.008$), and less frequently in well-differentiated tumors than in moderately to poorly differentiated tumors ($P = 0.036$). There was no difference in the postoperative survival between low and high MAGE-D4 patients (5-year survival rates, 65% and 69%, respectively; $P = 0.742$).
Conclusion: MAGD-D4 co-localized with beta-tubulin in a cell cycle specific manner and may play some roles in cell division. MAGE-D4 was correlated with tumor cell proliferation whereas MAGE-D4 status failed to have a prognostic value, suggesting that MAGE-D4 could be a molecular target for prevention and therapy of NSCLC.

1187 PUBLICATION

Combined therapy with radiation and S-1, an oral new 5-FU prodrug, is markedly effective against non-small cell lung cancer xenografts in mice

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Background: Radiochemotherapy is usually used to treat advanced (Stage III to IV) non-small cell lung cancer (NSCLC). Cisplatin (CDDP)-based regimens are often used for chemotherapy. S-1 is a novel oral 5-fluorouracil (5-FU) derivative that combines tegafur with Gimeracil, which inhibits the degradation of 5-FU in the liver and tumors, and oteracil, which reduces 5-FU-induced gastrointestinal toxicity. S-1 has been demonstrated to be active against advanced NSCLC. We investigated whether a combination of oral S-1 and tumor irradiation potentiated antitumor activity against human NSCLC xenografts in vivo.
Methods: Human lung cancer xenografts, designated LC-11 and Lu-99, were used. Tumor fragments were implanted *s.c.* into the right leg of nude mice. Two weeks later, the mice were treated with oral S-1 (8.3 mg/kg) once daily for 14 days or local tumor irradiation (2 Gy and 5 Gy) on day 1 and 8, or both. As reference regimens, CDDP (5 mg/kg, day 1) or UFT (tegafur plus uracil, 17.5 mg/kg for 14 days) was administered according to similar schedules in the same model. The effects of the treatment were evaluated on the basis of the delay in tumor growth.
Results: Combined treatment with S-1 plus 2 Gy tumor irradiation was significantly more effective against both lung tumors than S-1 alone and 2 Gy irradiation alone, and the effect of this combination was nearly equivalent to that of 5 Gy irradiation alone. A combination of S-1 plus 2-Gy irradiation was also more effective than the same dose of irradiation plus CDDP or UFT against LC-11 tumors. Gimeracil was found to potentiate sensitivity to lower-dose irradiation, whereas gimeracil alone had neither antitumor nor toxic activity, suggesting that this component of S-1 was involved in enhancing the response to irradiation.
Conclusion: Our preclinical results strongly suggest that radiochemotherapy with the new oral 5-FU derivative S-1 plus low-dose tumor irradiation would contribute to the improved treatment of patients with advanced NSCLC.

Melanoma and Sarcoma

Oral presentations (Mon, 31 Oct, 9.15–11.15)
Melanoma and sarcoma

1188 ORAL

Final data of the EORTC phase 1 study determining safety of Caelyx in combination with Ifosfamide in previously untreated adult patients with advanced or metastatic soft tissues sarcomas

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Background: Caelyx seems to have the same efficacy in adult soft tissue sarcomas as Doxorubicin with an improved toxicity profile. It could thus be an alternative to Doxorubicin and may well be easier to combine with agents such as Ifosfamide. The present phase 1 study evaluated the toxicity of combining Caelyx with Ifosfamide as 1st line treatment for patients (pts) with advanced and/or metastatic soft tissue sarcomas.
Material and methods: Eligibility included soft tissue sarcomas progressing within 6 weeks, no previous chemotherapy for advanced disease, WHO PFS <2, age 19–69 years and adequate cardiac, liver, renal and haematological function. Five dose levels were studied: Caelyx 30 mg/m^2 (level 1–4) or 40 mg/m^2(level 5) 1-h infusion d 1 q 3 w + Ifosfamide (with Mesna) at Xg/m^2/4-h d 1–3 q 3 w at 5 doses: Level 1: X = 1.7 g; level 2: X = 2 g; level 3: X = 2.5 g; level 4 and 5: X = 3 g. Cohorts of 3 pts were entered at each dose level unless a DLT occurred, defined as ANC $<0.5 \times 10^9$ lasting for 7 days or for 3 days + fever ($\geqslant$38.5 °C), grade 4 thrombocytopenia, any grade 3–4 toxicity except nausea, vomiting and alopecia, and any toxicity requiring a 2 w delay. In case of DLT in 1/3 pts a new cohort was added. Toxicity was evaluated by CTC. Non-evaluable pts were replaced.
Results: 28 pts have been included. Median age was 60 years (29–69). Four pts were included at dose level 1, 8 pts at level 2, 3 pts at level 3, 6 pts at level 4, and 7 pts at level 5. No DLT was observed at level 1–4. Four pts with DLT were observed at dose level 5: Febrile neutropenia, renal insufficiency, dyspnoea, confusion and allergy. Otherwise toxicity was generally acceptable and primarily granulocytopenia and leucopenia. Non-haematological toxicities >grade 2 were few. PPE >grade 1 was not seen. At present response is evaluable in 19 pts of which 2 obtained PR and 13 SD.
Conclusions: Combined Caelyx and Ifosfamide seems to be feasible in pts with advanced soft tissue sarcomas allowing administration of Ifosfamide at a dosage similar to that used when Ifosfamide is given alone. The recommended dose is Caelyx 30 mg/m^2/1-h d 1 + Ifosfamide (with mesna) at 3 g/m^2/4-h d 1–3 q 3 w.