of proficient *CYP1A1* genotype with deficient *GSTM1* variant would result in particularly elevated lung cancer (LC) risk, especially for squamous cell carcinoma (SCC).
Material and methods: In order to validate whether the *CYP1A1-C*^{3801} (*CYP1A1*2*) allele has an unfavorable significance alone and/or in combination with the *GSTM1* deficiency, we compared the genotype distribution in LC patients (n = 141), healthy donors (HD, n = 204), and elderly tumor-free smokers and non-smokers (ED, n = 246).
Results: *CYP1A1*2* allele carriers demonstrated a clear-cut association with SCC: the adjusted OR were 2.22 (95% CI = 1.06–4.63) and 2.27 (95% CI = 1.14–4.52) when HD and ED were used as referents, respectively. *CYP1A1*2(+)/GSTM1(−)* combined genotypes were overrepresented in the SCC patients (14/70, 20.0%) and underrepresented in the ED (19/246, 7.7%) as compared to the intermediate prevalence in the HD (26/204, 12.7%); the adjusted OR for SCC versus ED reached 3.85 (95% CI = 1.43–10.33).
Conclusions: In agreement with some literature data, our results support the concerted role of *CYP1A1* and *GSTM1* at-risk genotypes in SCC predisposition.

1178 PUBLICATION

Nuclear Factor-kappa B activation by TNF-alpha in mesothelial cells and expression in Malignant Mesothelioma

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Background: Nuclear Factor-kappa B (NF-κB) is a heterodimeric transcription factor central to cellular stress responses and protection against apoptosis, making it a potential target for novel anti-cancer therapies. The role of NF-κB in malignant mesothelioma (MM) is not clear.
Materials and Methods: We evaluated 1) the nuclear translocation of NF-κB in MET5A cells in response to TNFα by immunofluorescence and a nuclear protein factor p65 assay; 2) the degradation of IκBα in response to TNFα, with and without the administration of pharmacological inhibitors; 3) the expression of NF-κB by immunohistochemistry in 146 MM samples and its impact on survival. NF-κB expression was correlated with clinicopathological variables, tumour angiogenesis and necrosis and the expression of the Epidermal Growth Factor Receptor (EGFR). The impact of NF-κB expression on survival was determined.
Results: The pattern of NF-κB expression in untreated MET5A cells was cytoplasmic, with nuclear translocation occurring in response to TNFα administration. Significantly increased levels of nuclear p65 were noted at 8 and 24 hours. Degradation of IκBα was observed in MET5A cells in response to TNFα, but this was not altered by the administration of LY294002, U0126, SB20380, NS398, Iressa or vitamin E. Although cytoplasmic or membranous immunostaining was seen in the majority of tumour samples (96.5%), nuclear localisation of NF-κB was seen in only 11% cases. There was no significant correlation between the level of expression of NF-κB and standard clinicopathological prognostic factors. NF-κB correlated with the expression of EGFR ($p = 0.001$). Survival analysis showed that nuclear NF-κB expression was associated with reduced survival ($p = 0.04$), whereas cytoplasmic expression was not ($p = 0.7$).
Conclusions: NF-κB is activated in MET5A human mesothelial cells in response to TNFα. NF-κB expression is a common feature of MPM and may be a novel prognostic factor. NF-κB may play an important role in the carcinogenesis of MM. NF-κB may be a valid therapeutic target for novel therapies in MPM.

1179 PUBLICATION

EGFR mutation in lung cancers treated by Gefitinib in Thailand

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Background: The sub-group analysis of IDEAL 1 and IDEAL 2 gefitinib studies interested us in that the objective response rate (ORR) of Japanese NSCLC patients fared better than that of Caucasian counterpart. We started our work in Thailand recently by using the mutation study of EGFR gene reported recently by Lynch et al and Paez et al. We ask two questions: 1) Do gefitinib responders of THAI Ethnicity have EGFR mutations as seen in other studies? 2) Is the EGFR mutation rate in THAI NSCLC patients with no gefitinib treatment higher than that of international standard ?
Materials: Fresh lung tumor tissues and or tissue paraffin blocks of six Thai NSCLC gefitinib responders were studied by DNA extraction followed by PCR and DNA Sequencing. Normal DNA pair of each patient was obtained from their own blood leukocytes. DNA from 4 NSCLC patients of our own study who have yet to start gefitinib treatment and 10 additional DNA samples from NSCLC Archives tissue paraffin blocks and from frozen tissue bank were studied for the baseline EGFR mutation rate.
Results:
1. All six gefitinib responders have EGFR gene mutations 6/6 (100%).
2. Deletions and point mutations were among the most commonly found events, however, the base insertions have also been found often in exon 21.
3. Our preliminary data of DNA from 12 NSCLC samples without treatment have mutation rate of 4 /14 (28.5%) in their EGFR genes, exons 19 and 21.

Conclusion:
1. For Non small cell lung cancer, EGFR gene mutations at the Tyrosine Kinase Domain appeared to be required for objective gefitinib response.
2. Our preliminary data, even still small in number, appeared to suggest the high mutation rate in NSCLC in Thai Ethnic patients. Perhaps, this could explain the high success rate of gefitinib treatment in Asian countries. Further study is needed to substantiate our findings.

1180 PUBLICATION

The role of TTF1 as prognostic factor in stage III non-small cell lung cancer (NSCLC)

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Background: The 1997 International Staging System (ISS) separated stage III NSCLC patients into IIIA and IIIB. Stage III NSCLC represents a heterogeneous group and the ISS remains unsatisfactory in term of prognosis prediction. In a previous study, we observed that survival was better predicted when unresectable NSCLC patients were classified into stages IIIβ (T3–4N3) and IIIá (other TN stage III). The aim of the present study was to determine the role of a biological factor, TTF1 (thyroid transcription factor 1) as prognostic factor in stage III NSCLC in addition to stage and other known clinical factors.
Material and methods: All stage III NSCLC patients treated in our hospital were retrieved and searched for biopsy specimens. TTF1 was assessed by immunohistochemistry (Novocastra SPT24).
Results: Between 01/1987 and 07/2003, 108 assessable stage III NSCLC patients, for whom biopsies were available, were included in the study. Their principal characteristics were: median age 64 years (range 37–83), male/female 81/27, squamous/non squamous 52/56, IIIA/IIIB 44/64, III/III 89/16, median Karnofsky PS 80 (range 20–100). They were treated according to the following modalities: chemotherapy alone 44, radiotherapy alone 15, surgery alone 3 and combined treatment 46. Forty-four patients were positive for TTF1 (squamous 25.0% vs non-squamous 55.4%; $p = 0.007$). Nineteen patients were alive at the time of analysis (05/2005). In univariate analysis, good PS, surgery, normal platelet count were found good prognostic factors for survival ($p < 0.05$). In multivariate analysis, including all variables with a p value less than 0.2 in univariate analysis, only 3 factors were statistically significantly associated with better survival: good PS ($p = 0.005$), surgery ($p = 0.004$) and creatinine level ($p = 0.02$). When the analysis was restricted to adenocarcinoma or to non-squamous histology, TTF1 was found a potential prognostic factor for survival in univariate analysis ($p < 0.05$).
Conclusion: In stage III NSCLC patients, good PS, resectability and low creatinine level but not TTF1 are prognostic factors for survival. Nevertheless, TTF1 appears a potential prognostic factor for survival in adenocarcinoma.

1181 PUBLICATION

Bcl-2 family proteins and lymph node metastasis in bronchopulmonary carcinoid tumors

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Apoptosis or programmed cell death is a regulated process responsible for deletion of single cells in normal tissue turnover, allowing the organism to tightly control cell numbers and tissue size. The Bcl-2 family of proteins, which has a crucial role in intracellular apoptotic signal transduction, is composed by pro-apoptotic (Bax) and anti-apoptotic (Bcl-2) members. The objective of this study was to survey the occurrence of apoptosis in

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