50 μM LY in both cell lines. Both cell lines expressed Bcl-X (L) and Bcl-2, but the effect of LY was ambiguous.
Conclusions: Effective inhibition of P-Akt corresponded to an increased apoptosis in wt cells, but not to caspase activation. In res1.2 cells, efficient inhibition of P-Akt never occurred, and there was no change in apoptosis. The inefficient inhibition of P-Akt by LY indicated that PI3-kinase is either strongly overactivated or that PI3-kinase is not the main phosphorylator of Akt 1 in P31 cells. The role of the anti-apoptotic proteins Bcl-X (L) and Bcl-2 in survival of P31 cells needs to be further investigated. To conclude, P31 res1.2 cells appeared to be more resistant to LY inhibition of P-Akt than P31 wt cells. In P31 wt cells, P-Akt was important for survival and affected caspase-3 activity. The involvement of PI3-kinase and Akt/PKB in the cisplatin-induced apoptosis signalling pathways of P31 wt and res1.2 cells remain to be elucidated.

1155 POSTER

EGFR mutations in NSCLC: Genotypic analysis and implementation of complementary screening tests for detection purposes

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Background: Somatic mutations of the epidermal growth factor receptor (*EGFR*) gene in non-small cell lung cancer (NSCLC) predict responsiveness to the EGFR tyrosine kinase inhibitors. These mutations are commonly identified using DNA sequencing methods. Although considered the gold standard, this approach requires a high ratio of tumor to normal tissue DNA for optimal results which is not often available in biopsies obtained from these patients. Due to this limitation, we have applied selected screening tests to enhance the sensitivity of DNA sequencing.
Materials and methods: Clinical specimens from 50 NSCLC patients were analysed for *EGFR* mutations in exons 18, 19, and 21. After DNA extraction and PCR, mutations were examined by sequencing genomic DNA. Additionally, PCR products were screened for exon 19 deletions using a fragment analysis strategy.
Results: Sequencing revealed 5 mutations: 3 missense mutations in exon 21 and 2 deletion mutations in exon 19. Fragment analysis of the samples detected the original 2 deletion mutations and an additional 4 new exon 19 deletion mutations that were further confirmed by direct sequencing with re-designed PCR primers. In our hands, fragment analysis was able to detect mutations in samples containing as little as 10% mutated DNA whereas direct sequencing requires at least 30%.
Conclusion: Clinically relevant mutations in the *EGFR* gene may not be detected using sequencing techniques because of insufficient tumor DNA in biopsy samples. The application of additional rapid and more sensitive screening tests may be able to overcome this limitation. Fragment analysis is a quick and reliable method for the detection of *EGFR* exon 19 deletion mutations in lung cancer that may be missed by standard DNA sequencing methods. Fragment analysis to detect deletion mutations and other more sensitive screening tests to detect missense mutations should be implemented as complimentary methods for detection of *EGFR* mutations.

1156 POSTER

Identification of prognostically significant subsets of stage IIIA N2 non-small cell lung cancer patients by hierarchical clustering analysis of tissue microarray immunostaining. An Alpe-Adria Thoracic Oncology Multidisciplinary group study (ATOM 014)

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Background: Based on gene expression profiling, prognostically relevant subsets of patients have been identified for breast cancer, lung cancer and lymphoma. In the future, gene expression profile of each individual patient might provide support for tailored therapeutic decision making.
Methods: We performed a hierarchical clustering analysis of tissue microarray (TMA) immunostaining data of 87 patients with stage IIIA pN2 non small cell lung cancer (NSCLC), treated with radical surgery between 1985 and 1997. The expression of the following markers was evaluated: EGFR, ErbB-2, c-kit, COX-2, survivin, bcl-2, cyclin D1, cyclin B1, MMP-2, MMP-9 and univariate, multivariate analyses and unsupervised hierarchical clustering analysis by using these 10 markers were performed.
Results: Bcl-2 ($p < 0.0001$) and cyclin D1 ($p = 0.0036$) are more expressed in squamous cell carcinoma (SCC), while MMP-2 ($p = 0.0115$), MMP-9 ($p = 0.0075$) and survivn ($p = 0.02$) display increased expression levels in histological subtypes other than SCC. In univariate analysis, only squamous cell histology, bcl-2 and cyclin D1 expression were favorable prognostic factors ($p = 0.0149$, $p = 0.0013$, $p < 0.0001$, respectively), while MMP-2 expression was associated with worse prognosis ($p = 0.013$). In multivariate analysis, cyclin D1 and MMP-2 were the only positive and negative prognostic factors, respectively ($p < 0.0001$, $p = 0.06$). Unsupervised hierarchical clustering analysis of TMA immunostaining data produced 5 distinct cluster groups and the deduced tree identified 2 prognostically significant subsets of patients, with better (groups 1–2) and worse (groups 3–4–5) prognosis in terms of median survival (51 vs. 10 months, $p < 0.0001$). Notably, groups 1–2 were mostly composed of SCC (80%).
Conclusions: These results suggest that hierarchical clustering of TMA immunostaining data by using a limited set of markers might provide a useful tool for the identification of radically resected NSCLC patients at high risk of recurrence, likely to benefit from more aggressive treatment.

1157 POSTER

Expression of hypoxia-inducible factor-1 alpha and its prognostic significance in small-sized adenocarcinomas of the lung

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Objective: To analyze the prognostic value of hypoxia-inducible factor-1 alpha expression and its correlation with clinicopathologic variables and expression of vascular endothelial growth factor-A, -C, and R-2 in patients with lung adenocarcinomas of small size.
Methods: The expression of hypoxia-inducible factor-1 alpha was immunohistochemically determined in 78 cases of small-sized adenocarcinoma (maximum dimension is less than 2 cm) using polyclonal antibody against a recombinant protein corresponding to amino acids 575–780 of hypoxia-inducible factor-1 alpha. Data regarding patient survival, clinicopathologic factors, and immunohistochemical studies of vascular endothelial growth factor were also collected.
Results: Strong expression of hypoxia-inducible factor-1 alpha was observed in 29 (37%) of 78 cases; no expression was found in the bronchioalveolar carcinomas. Strong expression of hypoxia-inducible factor-1 alpha was significantly higher in cases with vascular invasion, lymphatic permeation, lymph node involvement, and advanced pathological stage. Strong expression of hypoxia-inducible factor-1 alpha was correlated with strong expression of vascular endothelial growth factor-A, -C, and R-2. The 5-year survival rate was 69% if expression of hypoxia-inducible factor-1 alpha was strong and 84% if expression was weak. Multivariate analysis revealed that pathological N status and pleural invasion were independent prognostic factors and strong expression of hypoxia-inducible factor-1 alpha was marginal significance.
Conclusions: Strong expression of hypoxia-inducible factor-1 alpha was associated with vascular invasion, lymphatic permeation, nodal involvement, pathological stage, and strong expression of vascular endothelial growth factor-A, -C, and R-2. Strong expression of hypoxia-inducible factor-1 alpha was a poor prognostic factor for patients with small-sized adenocarcinoma of the lung.

1158 POSTER

Can 18FDG-PET/CT scan be used to define a biological target volume (BTV) for IMRT treatment planning of non-small cell lung cancer patients?

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Purpose: To test the feasibility of FDG based PET/CT data on target volume delineation in radiotherapy treatment planning of NSCLC patients, and impact of these outlined biological target volumes (BTV) for IMRT treatment.
Materials and methods: Patient diagnosed with non-operable NSCLC in the right upper lobe had a 3D conformal planning based on CT data with our hypo-fractionated regimen of 52.5 Gy in 15 fractions. Planning was