available) were obtained at the time of surgical resection at Tokyo dental college Chiba Hospital after informed consent had been obtained from the patients according to a protocol that was approved by the institutional review board of Tokyo Dental College.
Result: Using quantitative real-time reverse transcription polymerase chain reaction, western blotting and immunofluorescence on seven OSCC-derived cell lines and NOKs, *Syk* mRNA and protein expression were commonly down-regulated in all cell lines compared with the NOKs. Although no sequence variation in the coding region of the *Syk* gene was identified in these cell lines, we found frequent hypermethylation in the CpG island region. Syk expression was restored by experimental demethylation. In addition, using a wound healing assay and in vitro invasion assay, we performed functional analysis using Syk transfected into the OSCC-derived cell lines, and they showed significant inhibition of motility and invasiveness. In clinical samples, high frequencies of Syk down-regulation were detected by immunohistochemistry (33 of 53 [62%]). Furthermore, the Syk expression status was correlated significantly ($P = 0.047$) with tumor metastasis to cervical lymph nodes.
Conclusion: These results suggest that the *Syk* gene is frequently inactivated during oral carcinogenesis and that an epigenetic mechanism may regulate loss of expression possibly leading to metastasis.

1335 POSTER

Vascular endothelial growth factor (VEGF) 936 C/T gene polymorphism is a risk factor for invasive ductal carcinoma of the breast in a sample of Croatian woman

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Cancer angiogenesis development mediatet thru vascular endothelial growth factor (VEGF) has important role in cancer metastasing and malignant growth. The aim of this study was to investigate potential relationship between VEGF gene 936 C/T polymorphism and coexistance of invasive ductal breast carcinoma in a sample of Croatian woman. In addition, there is no any data about described genetic polymorphism among Croatian feemale population. We enrolled two groups of feemale patients: 122 subjects with invasive breast carcinoma (mean age 54.1±4, range 36−81 years) and 156 healthy control subjects (mean age 57.4±6, range 32−75 years) without any history of malignancy in which the clinical evaluation including mammography and breast ultrasound did not reveal any breast pathology. Genomic DNA was isolated from peripheral venous blood, while single nucleotide polymorphism 936 C/T genotyping in the VEGF receptor was performed using PCR-RFLP methode. We have not detected any 936 T/T genotype of VEGF gene but significant association of breast cancer risk was shown in the group of woman with breast invasive ductal carcinoma compared to healthy group. Carriers of the 936 C/T genotype were more frequent among woman with invasive ductal carcinoma (46 of 122 examinies, 37.7%) than among control group (7 of 156 examinies, 4.5%). The difference was statisticaly significant ($p < 0.0001$). This study found significant evidence that examined gene polymorphism is a key factor associated with susceptibility to invasive ductal carcinoma of the breast in a sample of Croatian women.

1336 POSTER

The prognostic impact of serum angiogenic factors in renal cell carcinoma

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Background: To determine the selected serum angiogenic factors in patients with newly diagnosed renal tumor (RCC) and to correlate them with the clinical stage of the disease.
Patients: Nephrectomy or partial kidney resection was performed in 54 patients (34 men, 20 women) with newly diagnosed renal cell carcinoma (RCC). The mean age of patients was 64.4 years. The patients were divided into three groups based on the TNM classification: the 1st group – stages I + II, the 2nd group – stage III, the 3rd group – stage IV.
Method: The serum levels (collected before surgery) of GROα (CXCL1), IL-8 (CXCL8), IL-6, VEGF and bFGF were determined by the ELISA method. Clinical data (age, sex, tumor histopathology grading (HPG), disease progression and death during a-12-month follow-up period) were compared with serum levels of angiogenic factors. Kruskal-Wallis ANOVA, Mann-Whitney's test and Kolmogorov-Smirnov's test were used.
Results: In case of GROα, a significant difference was found between patients with and without progression period ($p = 0.006$), between surviving and dead patients ($p = 0.038$), between the1st and the 4th grade of HPG ($p = 0.05$), between the 2nd and the 4th grade of HPG ($p = 0.0043$) and between the 3rd and the 4th grade of HPG ($p = 0.044$). A statistically significant difference in serum concentration of IL-8 was found between patients with and without progression ($p = 0.007$), but no differences were found between the dead and surviving patients and between the various grades of HPG. A statistically significant differences in serum concentration of IL-6 were found between patients with and without progression ($p = 0.0006$) between dead and surviving patients ($p = 0.0042$), between the1st and the 4th grade of HPG ($p = 0.05$) and between the 2nd and the 4th grade of HPG ($p = 0.0041$). A statistically significant difference in serum levels of VEGF was found between patients with and without progression ($p = 0.0026$), between dead and surviving patients ($p = 0.021$). A statistically significant difference in serum levels of bFGF was found between dead and surviving patients ($p = 0.024$).
Conclusion: Out of the ten tested angiogenic factors, we found a correlation between serum levels and clinical findings for GROα, IL-8, IL-6, VEGF and bFGF. However, their use in clinical practice should be verified on a larger group of patients.
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1337 POSTER

Free light chains renal handling in patients with plasma cell dyscrasias

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Introduction: Serum immunoglobulin-free light chain (FLC) assay is a major marker in the evaluation and management of patients with plasma cell dyscrasias. In a number of these patients, anomalies in monoclonal FLC may induce tissue deposition and diseases, especially in kidney. We retrospectively analyzed the renal handling of FLC in different types of plasma cell dyscrasias.
Methods: K and L FLC concentrations were measured by nephelometry (Freelite ®, The binding site) in serum (s) and 24-h urine (u) from 85 patients. 11 patients had polyclonal hypergammaglobulinemia (H) but no monoclonal protein as detected by protein immunofixation electrophoresis (IFE) in serum and urine. 74 patients presented abnormal K/L FLC ratio (rFLC) in serum and/or urine. 22 of them had multiple myeloma (MM) with monoclonal intact immunoglobulin or FLC only, with K (MMK, n = 13) or L (MML, n = 9) light chain. 52 of them, without MM, had an increased (I) or a decreased (D) rFLC, and monoclonal protein detected by sIFE and/or uIFE.
Results: See the table.

	H	MMK	MML	I1	I2	D1	D2
CK ml/min	2.7 (0.6–6.6)	1.8 (0.9–12)	3.0 (1.9–12)	1.4 (0.4–7.3)	0.2 (0.1–0.4)$^{£}$	1.7 (0.4–4.9)	0.7 (0.2–3.9)
CL ml/min	0.6 (0.1–1.5)	1.2 (0.1–2.5)	0.8 (0.5–5.0)	0.2 (0.1–2.9)	0.1 (0.1–1.2)	0.8 (0.1–2.0)	0.02 (0.01–0.1)$^{\$}$
creatinine µmol/l	177 (112–461)	162 (63–559)	221 (72–799)	134 (60–730)	121 (61–278)	133 (64–474)	139 (60–220)

Values are median (ranges); Mann-Whitney test (significance: P < 0.05; *:MM vs H; $^{£}$: I1 vs I2; $^{\$}$:D1 vs D2).

In H, MMK and MML groups, comparable for glomerular filtration, renal clearance of K FLC (CK) and L FLC (CL) were similar indicating similar FLC renal handling in these patients with mono- or polyclonal diseases. I group was splited in 2 groups according to CK: in I2 (n = 11) as compared to I1 (n = 24) group, CK was decreased (P < 0.0001) and sFLC increased [343 (54–817) vs 47 (20–285)$^{£}$ mg/l]. D group also was splited in 2 groups according to CL: in D2 (n = 7) as compared to D1 (n = 10) group, CL was decreased (P < 0.002) and sFLC increased [213 (150–416) vs 90 (28–247)$^{\$}$ mg/l]. Low CK and CL values were also significantly decreased in I2 and D2 groups as compared to MMK, MML and H groups and as compared to patients without plasma cell dyscrasia, regardless of creatinine (not shown). Strikingly, in I2 group, 1 light chain deposition disease and, in D2 group, 2 AL-amyloidosis were diagnosed while 4 AL-amyloidosis were diagnosed in D1 group. Such low FLC renal clearance was also observed in 3 cases of MM (not included here).
Conclusions: Low FLC renal clearance might result from FLC renal tissue deposit or FLC aggregation reducing renal excretion. Whether FLC renal

clearance is a marker of pathogenicity, diagnosis or prognosis in plasma cell dyscrasias requires further study.

1338 POSTER

Identification of potential diagnostic markers in bronchial fluid of patients with non small cell lung cancer (NSCLC)

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Background: Lung cancer ranks among the most common and most lethal malignancies worldwide. Given the fact that survival of lung cancer patients is very poor, it is logical to speculate that early detection might result in more favourable outcomes for these individuals. New proteomic techniques can identify potential diagnostic and prognostic markers. The aim of this study was to find protein markers in bronchial fluid which could enable early diagnosis in NSCLC.
Materials and Methods: We have included 96 patients with NSCLC diagnosed using bronchoscopy (64 scamous/29 adenocarcinoma/3 others) and 49 consecutive patients with non pathological bronchoscopy. Bronchial fluid was obtained from each patient and potential protein markers were studied. Bronchial fluid was centrifuged and supernatant proteins were analysed using bidimensional electrophoresis with poliacrilamid gel stained with silver nitrate. Gel was scanned and analysed with *Progenesis* PG6220 program, which measures intensity of each spot. Resultant intensities in each group of patients (NSCLC/non pathological bronchoscopy) were compared using T-Student method. We selected as potential markers those spots with a p value of less than 0.05. We calculated "fold change" of each spot as the ratio between mean intensity in NSCLC bronchoscopies samples and non pathological bronchoscopies samples.
Results: We analysed 300 spots in each sample and we found 31 potential markers whose fold-change ranges from 1.49 to 7.41; 15 of the markers were expressed in a higher level in NSCLC samples and the other 16 were expressed in a lower level.
Conclusions: We have identified 31 differential protein markers in bronchial fluid among our patients. These results could lead in an early diagnostic test which must be validated in future studies.

1339 POSTER

Free light chains in patients with renal impairment associated or not with hypergammaglobulinemia

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Introduction: Serum immunoglobulin-free light chain (FLC) assay is a major marker in the evaluation and management of patients with plasma cell dyscrasias. However FLC are known to be metabolized by kidneys and these patients frequently present renal insufficiency. We retrospectively assessed the effect of renal impairment on serum and urinary polyclonal FLC values in patients with or without polyclonal hypergammaglobulinemia.
Methods: K and L FLC concentrations were measured by nephelometry (Freelite ®, The binding site) in serum and 24-h urines from 80 patients [73 (22−91) years]. Patients with monoclonal protein detected by serum and/or urinary protein immunofixation electrophoresis were excluded. Three arbitrarily groups of patients with normal serum immunoglobulins concentrations and FLC K/L ratio (rFLC) were defined with respect to type of proteinuria (Hydragel Proteinuria, Sebia) and to K and L FLC renal clairance ratio (CK/CL) as physiologic (C, n = 11), predominant tubular (T, n = 27) or predominant glomerular (G, n = 31) groups. A fourth group of patients had renal impairement and polyclonal hypergammaglobulinemia (H, n = 11). Results: median (ranges); Mann-Withney test (significance: $P < 0.05$), Spearman correlations (significance: $P < 0.02$).
Results: Throughout C, T and G groups, K and L FLC serum concentrations, urinary excretions and renal clearances were significantly inversely correlated with glomerular filtration (as evaluated by 1/serum creatinine concentration), regardless of the type of proteinuria. Serum rFLC was inversely correlated while urinary rFLC and CK/CL were positively correlated with glomerular filtration. In H as compared to G group, glomerular filtration and CK, CL, CK/CL were similar suggesting similar renal impairment; however, in H as compared to G group, FLC serum concentrations of K [101 (57–344) vs 34 (20–120) mg/L, respectively] and L [60 (42–174) vs 34 (15−78) mg/L, respectively], urinary excretions of K [327 (91–1328) vs 140 (35–537) mg/24 h, respectively] and L [57 (24–371) vs 40 (10–252) respectively] and serum rFLC [1.3 (1.0−2.3) vs 1.0 (0.4−1.7) respectively] were significantly higher.
Conclusions: K and L FLC serum concentrations, urinary excretions and serum rFLC increased with progressive renal impairment, an effect that was reinforced by polyclonal hypergammaglobulinemia. Therefore, interval references for K and L values and serum and urinary rFLC probably should be related to creatinine for the evaluation and management of patients with plasma cell dyscrasias presenting renal insufficiency with or without polyclonal hypergammaglobulinemia.

1340 POSTER

Study of EGFR mutation expression in adenocarcinoma of lung and their implication in the specific treatment – institutional experience

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Background: In the last years, the work groups that are contributing to the study of the treatment of the non-small-cell lung cancer (NSCLC), being one of the emergent lines, the epidermal growth factor receptor (EGFR). Retrospective analyses of the biopsies of patients, mutation of the tyrosine kinase domain of the EGFR confer to the patients a strong sensitivity to gefinitib.
Purpose: Examine retrospectively EGFR mutations at exones 18, 19 and 21 to evaluate the prevalence in a small series of our institution with pulmonary confirming biopsy of adenocarcinoma.
Materials and Methods: We studied 23 patients, of them 7 were females an 16 males, with median age was 58 years (32−76). The stage of patients at diagnosis, according the TNM staging system was: 9 patients (39%) were classified at stage I; 1 (4.5%) at stage II; 7 (30.5%) at stage III; and 6 (26%) at stage IV. Depending on personal background, 20 patients (87%) were smokers and the rest (13%) non-smokers. The study took place on samples peripheral blood and paraffin-embedded tissue. The analysis of gene that codifies the EGFR made by means of genomic obtaining of DNA: 1) from blood-EDTA, 2) from the rich paraffin tissue in tumour. The exones identification 18, 19, and 21 of gene EGFR was made by means of PCR. In each series two controls were used: one positive with genomic DNA of a patient control and one negative (without DNA) to detect possible contaminations. The patients previously were informed about the test that was going away to make, following the effective ethical norms in our country.
Results: Mutations were not detected DNA from peripheral blood of the 23 studied cases. Sixteen of 23 (69%) patients harbored mutations in EGFR gene. 4/23 (17%) presented prognostic therapeutic meaning mutations according to those described before, and were 3 cases of deletion LREA in exon 19 and one mutation L858 of exon 21. These 4 cases corresponded to females.
Conclusions: In our environment, the mutations frequency of EGFR gene in adenocarcinoma pulmonary with therapeutic-clinical meaning is very low and predominantly in female sex.
The systematic study of the EGFR gene mutations may allow the individualization of therapy for patiens with lung adenocarcinoma.

1341 POSTER

Potential diagnostic markers in bronchial fluid of small cell lung cancer (SCLC)

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Background: Lung cancer is a major cause of mortality worldwide and overall survival rate has not improved significantly over the past 20 years. Although the incidence of SCLC is declining, it remains a worldwide public health problem. An early diagnosis could improve prognosis and survival among these patients. The aim of this study was to identify protein markers obtained from bronchial fluids of SCLC patients which may differ from non-pathological bronchoscopy samples.
Materials and Methods: We have included 43 patients with SCLC diagnosed using bronchoscopy and 49 consecutive patients with non pathological bronchoscopy. Bronchial fluid was obtained from each patient and potential protein markers were studied. After being centrifuged, supernatant proteins were analysed using bidimensional electrophoresis