Conclusions: It is suggested that decreased expression of RECK gene have a role in the increase of MMP activity in osteosarcoma. Further study is required to analyze the mechanism of RECK action. It can be a new therapeutic strategy for MMP inhibition in human cancer.

1204 POSTER

Heparanase expression in melanoma: updated clinico-pathologic results.

R. Abdah-Bortnyak[1], N. Ilan[2], V. Kaplan[2], S. Billan[1], O. Ben-Itzhak[1], I. Vlodavsky[2], A. Kuten[1]. [1]*Rambam Medical Center, Oncology, Haifa, Israel;* [2]*The Bruce Rappaport Faculty of Medicine, Laboratory of Cancer Biology and Angiogenesis, Haifa, Israel*

Background: No effective systemic treatment exists for advanced melanoma. Identification of new markers involved in the initiation and progression of melanocytic tumorigenesis, that will be the basis for developing new therapeutic tools are still needed. The expression of the heparanase gene and its protein has been associated with metastatic potential of several human tumors. The purpose of the study is to determine the expression of heparanase in nevus and melanoma in different stages of tumor progression and to evaluate the clinico-pathologic significance of these findings.
Methods: 60 formalin-fixed and paraffin-embedded specimens of nevus (15) and melanoma (45) were examined with immunohistochemical staining for heparanase expression. The charts of all melanoma patients were reviewed for clinical correlation.
Results: No (0) heparanase expression in 7 specimens or weak (1+) in 8 specimens was detected in nevus. Heparanase was detected in both the cytoplasm and the nucleus of heparanase positive cells. Weak heparanase expression was confirmed in 11 specimens, weak to intermediate in 2 and intermediate (2+) in 2 specimens of superficial spreading melanoma (Breslow <4 mm). Intermediate intensity staining (2+) was detected in 14 cases of thick melanoma (Breslow >4 mm) and only one case showed weak staining. Strong (3+) heparanase expression predominated in 10 cases from different metastatic sites and intermediate to strong staining in 5 specimens (3 from lymph nodes and 2 from lung metastases). Of 15 patients with superficial spreading melanoma, 2 (13%) developed recurrent disease, at 4 and 5 years from diagnosis. In both patients heparanase expression at the time of diagnosis was weak: 1+. Of 15 patients with thick melanoma, 10 (67%) developed recurrent disease, and 6 (40%) died of melanoma. Heparanse expression at the time of diagnosis in this group of patients was intermediate in all patients who developed recurrence. Six of 15 patients (40%) with metastatic melanoma, who died from the disease, had strong and 3 patients had intermediate-to-strong expression of heparanase in tissue specimens obtained from metastases.
Conclusions: Heparanase expression in melanoma is significantly correlated with tumour stage and metastatic potential. The value of heparanse activity as predictor of clinical course of disease requires further investigation in a larger number of patients

1205 POSTER

Clinical significance of serum 5-S-cysteinyldopa determination in patients with malignant melanoma

M. Boldizsar, K. Gilde, T. Banfalvi, T. Kremmer, B. Vincze. *National Institute of Oncology, Biochemistry, Budapest, Hungary*

The incidence of malignant melanoma is increasing worldwide and the metastatic ability of the disease is very high. Among circulating tumour markers in melanoma (S-100B protein, neuron specific enolase, LDH, cytokines, tyrosinase, etc.) 5-S-cysteinyldopa (5-SCD) is extensively investigated, and may have potential role in the follow-up of patients, monitoring the therapy, prediction of prognosis and in the early detection of recurrences. 5-SCD is a precursor of pheomelanin produced in melanocytes and melanoma cells during the biosynthesis of melanins by a tyrosinase dependent mechanism. The purpose of this study was to evaluate the significance of this marker in the clinical practice by measuring the serum 5-SCD concentrations in different stages of malignant melanoma and monitoring the patients during the therapy, as well as to analyse the data concerning the progression of disease.
Since 1997, nearly 4500 serum samples originated from 1409 patients suffering from malignant melanoma were investigated. The age of patients (including 677 males and 732 females) ranged from 18 to 86 years (mean 56.7). The diagnosis of malignant melanoma and the presence of metastasis were verified by histology and by various imaging techniques. Serum 5-SCD concentration of healthy individuals and melanoma patients was determined by high pressure liquid chromatography with electrochemical detection. Patients were classified according to their AJCC Stages and data statistically evaluated. In addition, 180 patients (3 in Stage I, 93 in Stage II, and 84 in Stage III) were monitored for years.
Significant differences were revealed between control group and stage III-IV, as well as between stage III and IV patients. In about 25 percent of patients suffering from various types of recurrence the elevated 5-SCD level was the first sign of the progression. The increase of 5-SCD level preceded by 1–3 months the detection of spreading of the disease compared with conventional imaging methods.
Summarising our observations it was confirmed that serum concentration of 5-SCD correlates well with Stages in melanoma patients and progression of the disease. The marker had the greatest clinical significance in stage IV and showed important positive predictive value. According to the results presented here determination of serum 5-SCD concentration proved to be a useful tool in the monitoring of melanoma patients.

1206 POSTER

A study of familial melanoma in Greece and identification of germline mutations in the *CDKN2A* tumour suppressor gene

S. Retsas[1], H. Linardou[2], S. Murray[2], D. Bafaloukos[2], C. Andreadis[3], R. Tuominen[4], J. Hansson[4]. [1]*Cromwell Hospital, Oncology, London, UK;* [2]*Metropolitan Hospital, Oncology, Athens, Greece;* [3]*Theageneion Hospital, Thessaloniki, Greece;* [4]*Karolinska Institute, Stockholm, Sweden*

Background: The *p16/CDKN2A* tumour suppressor gene has been recognised as an important predisposing factor in the development of melanoma. The primary objective of this study is the identification of mutations in the *CDKN2A* gene among Greek families with at least two first-degree relatives afflicted by melanoma.
Material and methods: Members of such families with histological diagnosis of melanoma are invited to participate in the study. After informed written consent patients provide a blood sample. The study has the approval of the relevant Ethics Committee. Mutation analyses were performed on DNA isolated from peripheral blood and exons 1, 2, and 3 of the *CDKN2A* gene were amplified by PCR. All exons were bidirectionally sequenced.
Results: To date 12 Greek families have been identified who qualify for entry into the study. A total of 11 patients from 7 families and a relative with the *Atypical Naevus Syndrome* (ANS) have provided blood samples. Two or more members from 4 families and one surviving patient from each, of another 3 families have been studied. The Arg24Pro mutation in exon 1 has been identified in 6/11 patients who belong to 4 families. The Ala148Thr polymorphism in exon 2 and C500G in the 3'UTR have been identified in the relative with the ANS and in two patients, one of whom also has the Arg24Pro mutation. The study is ongoing.
Conclusions: The present study is the first systematic investigation of potential mutations in familial melanoma in Greece. The Arg24Pro mutation identified by us is likely to be of importance for melanoma risk, since it has previously been reported in different ethnic populations and been shown to segregate with melanoma. Our results indicate that this alteration may be the predominant *CDKN2A* germline mutation in Greek melanoma kindred.
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1207 POSTER

Oncogenes and tumor-suppressor genes in nodular melanoma. Prognostic value

A. Stanislawek, A. Kurylcio, L. Kuylcio, M.E. Krasuska, J. Mielko, W.P. Polkowski. *Medical University of Lublin, Surgical Oncology, Lublin, Poland*

The most important factors in prognosis of patients with melanoma are tumour thickness, presence of ulceration, localization, pathological stage, surgical treatment, depth of invasion, gender, and age. The purpose of the present study is to evaluate prognostic value of molecular markers (oncogenes, tumour-suppressor genes, apoptotic and proliferative factors, adhesion molecules) in patients with nodular melanoma. Tissue samples were obtained from 62 patients with nodular melanoma and a presence of ulceration, aged 34–81 years (male/female ratio – 1/1.38). Expression of p53, Bcl-2, Ki67, p21, C-myc, C-jun, Mdm2 and CD44 proteins was investigated immuno-histochemically in of primary melanoma lesion. Disease-free survival and overall survival were assessed. Logistic regression analysis was used to compare prognostic value of the proteins expression.
Results are presented in the table.
Conclusion: These results suggest that expression of p21, C-myc and CD44 in the melanoma cells might be additional prognosticators. Further studies are needed to investigate whether prognostic value of the molecular markers is independent of classical prognostic factors.