7008 POSTER

Locally generated elastin peptides increase invasive potential of melanoma cells dominantly by galectin-3

P. Pócza[1], A. Falus[1], Z. Darvas[1], K. Pócza[2]. [1]*Semmelweis University, Department of Genetics Cell- and Immunobiology, Budapest, Hungary;* [2]*Uzsoki Hospital, Department of Pathology, Budapest, Hungary*

Melanoma is a highly malignant tumor type and elastin protein plays a key role in the progression of melanoma. Melanomas containing more elastin are associated with higher Clark level, lymph node and distant metastases, greater tumor thickness and poor clinical outcome. The VGVAPG and VAPG peptide-sequences are repeating several times in the human elastin and they are most likely to be the breakdown products after the degradation of elastin.
We demonstrate the tropoelastin, elastin and the VGVAPG sequence with histochemical and immunohistochemical methods. We present evidence that both VGVAPG and VAPG elastin peptides could bind to three identical receptors: galectin-3, integrin $\alpha v\beta 3$ and elastin-binding protein.
We found significant changes of the expression of several metastatic markers in human melanoma versus normal skin samples. We investigated the effects of VGVAPG and VAPG elastin peptides on these metastatic markers in human melanoma cell lines with different invasive potential. Immunocytochemistry, flow cytometry and quantitative real-time RT-PCR were applied to evaluate the changes of the expressions.
In conclusion: interaction between phylogenetically conserved elastin sequences (VGVAPG, VAPG) and melanoma cells appears to be a significant point of tumor progression: (i) elastin and its fragments are potential substrates of MMP-2 and MMP-3; (ii) they have chemotactic effect on the melanoma cells; (iii) elastin peptides increase the expression of CXCR-4 and CXCL-12 chemokines; (iv) the cleaved peptide fragments have the ability to increase the expression of the elastin-degrading MMP-2 and MMP-3 enzymes; (v) they could increase the adhesion ability and the expression of CD44, ICAM-1 and NCAM-1 major adhesion molecules and (vi) increase the expression of the angiogenic VEGF-C. All these effects are mediated dominantly by galectin-3 receptor.

7009 POSTER

Antiproliferative activity of eugenol and curcumin related biphenyls on malignant melanoma cell lines

M. Pisano[1], M. Loi[1], M.G. Tilocca[1], G. Palmieri[1], G. Pagnan[2], D. Fabbri[1], M.A. Dettori[1], G. Delogu[1], M. Ponzoni[2], C. Rozzo[1]. [1]*Istituto di Chimica Biomolecolare, Progettazione Molecolare, Sassari, Italy;* [2]*Ospedale Pediatrico "Giannina Gaslini", Laboratorio di Oncologia, Genova, Italy*

Background: Malignant Melanoma (MM) is one of the most aggressive cancer and its incidence and mortality rates are highly increased during the last decades in fair skin populations. Chemotherapeutics currently in use are still unsatisfactory therefore the search for novel therapies is warranted. Eugenol and curcumin, main components of several spices, have both been described as potential anticancer agents. We tested several eugenol and curcumin-related compounds for their capability to inhibit cell growth on primary MM cell lines.
Material and Methods: Viability and antiproliferative assays together with dose and time-response assays have been carried out on MM cell lines to compare antitumour activity of both eugenol and curcumin to that of 11 related biphenyls. Cultured fibroblasts from healthy donors have been used as controls. TUNEL assays have been performed to asses apoptosis triggered by some of the treatments.
Results: Among the eugenol-related biphenyls, dibromo-dehydrodieugenol (S7) showed good antiproliferative activity on MM cells, being its enantiomeric form (−)-(S) the most active with IC50 ranging around 20−30μM and showing pro-apoptotic activity. S7-S treatments did not affect normal fibroblasts growth rate (M. Pisano et al., Mol Cancer 6:8, 2007). Curcumin, a natural occurring compound known for its antitumoral activity, showed potent antiproliferative activity on our MM cells (IC50 $\leqslant$ 10μM). We tested five curcumin-related hydroxylated biphenyls (D2-D6) on MM cells to assess their antiproliferative activity in comparison with curcumin itself and with the eugenol-related biphenyls. Preliminary experiments suggest that curcumin-related biphenyls are much more active in inhibiting MM cells growth comparing with the dibromo-dehydrodieugenol S7-S activity. One of them (D6) shows antiproliferative activity at concentrations lower than curcumin (IC50 around 1−2μM). Cultured human fibroblasts treated with D6 at the same concentrations were not affected in their proliferation rate. Work is on running to define doses and time of D6 action on MM cells and to investigate on pro-apoptotic activity.
Conclusions: Our results indicate eugenol and curcumin related compounds as good leads to develop new therapeutic agents against MM. Further investigations are needed to better define their antitumour activity and action mechanisms. Their activity should be then investigated on in vivo melanoma models to assess the real anticancer effectiveness on such tumour.

7010 POSTER

Characteristics of skin melanoma and determination of efficacy of its treatment by cytogenetic criteria

A. Monakhov[1], V. Anisimov[2]. [1]*NN Petrov Institut of Oncology, Group of Oncogenetic, St-Petersburg, Russian Federation;* [2]*NN Petrov Institut of Oncology, Department of Surgery, St-Petersburg, Russian Federation*

Background: Up to the present the earliest diagnosis and optimization of treatment methods are the main problems for skin melanoma. In this regard the purpose of this research was to find out an opportunity to diagnose this disease, to determine the degree of its malignancy, stage, prognosis, probability of metastasis, relapse or remission, and also treatment efficiency.
Materials and Methods: Cytogenetic investigation was carried out in lymphocytes of peripheral blood by the method described earlier (Monakhov A., Gulaev A., 1993). Cytogenetic investigation was performed before treatment, during different steps of treatment and after treatment in 67 patients with morphologically verified diagnosis of a skin melanoma stage I-III. 30 patients (group I) were treated only surgically. 7 patients (group II), were treated in the beginning with surgical method and then by polychemotherapy (vinblastin, dacarbazin, cisplatin). 30 patients (group III), were treated in the beginning with surgical method, then they were treated with immunotherapy by polyoxidonium (PO). PO – physiologically active highly-molecular compound with immunomodulating potency. Since 1996 PO has been allowed for medical use (registration N 96/302/9).
Results: Patients of the I−II groups had high level of cells with cytogenetic breaches (from 10 to 40%) almost through all periods of investigation. A stage were determined during background examination in all patients. The cytogenetic signs of metastasis were found out in 15 patients from group I, in 5 patients from group II and in 14 patients from group III. Attributes of secondary tumorous development were found out in 6 patients from group I, in 2 patients from group II and in 5 patients from group III. Specific cytogenetic markers for this disease, using which the diagnosis is possible, were revealed in 13 patient from the I group, in 3 patients from group II and in 17 patients from group III. The effect of the treatment was estimated in 5 patients of group I, in 2 patients of group II and in 21 patients of group III. The relapse's attributes of disease were determined in 23 patients of group I, in all patients of group II and in 7 patients of group III. The remission's attributes of disease were determined in 3 patients of group I, in 2 patients of group II and in 17 patients of group III.
Conclusions: Thus, considering cytogenetic criteria and health conditions, positive effectiveness of surgical method and next immunotherapy by PO. Cytogenetic data had prognostic value and correlated with clinical course of the disease. These data suggest that there is real possibility to diagnoze the disease, to characterize the process of its development, relapse or remission and to evaluate effectiveness of the therapy.

7011 POSTER

CDKN2A mutation in a Portuguese melanoma-prone family

A. Peixoto[1], C. Santos[1], P. Rocha[1], J. Vieira[1], M. Pinheiro[1], R. Henrique[2], R. Azevedo[2], F. Ribas[3], M.R. Teixeira[1]. [1]*Instituto Português de Oncologia do Porto FG EPE, Genetics, Oporto, Portugal;* [2]*Instituto Português de Oncologia do Porto FG EPE, Pathology, Oporto, Portugal;* [3]*Instituto Português de Oncologia do Porto FG EPE, Dermatology, Oporto, Portugal*

Background: Most melanomas arise as a result of a combination of genetic and environmental factors. Nearly 10% of cutaneous melanoma occur in a familial setting. CDKN2A germline mutations are found in 20 to 40% of families with three or more cases of melanoma and in about 2% of all melanomas. Pathogenic germline mutations in this gene are associated with a greatly increased lifetime risk of melanoma (up to 58% in UK, 76% in the USA, and 92% in Australia).
Materials and Methods: One family meeting the criteria for diagnosis of hereditary predisposition to melanoma received genetic counseling before and after genetic testing. Genomic DNA from an affected member of this family was prepared from peripheral blood lymphocytes using conventional procedures. Mutation screening of exons 1?, 1?, 2 and 3 of the CDKN2A gene was performed by automatic sequencing analysis. In order to predict the deleterious nature of the variant found in this family we combined segregation analysis, CDKN2A loss of heterozigosity (LOH), by FISH (fluorescence in situ hybridization) in tumor samples and in silico analysis using the SIFT (Sorting Intolerant From Tolerant) and the PolyPhen (Polymorphism Phenotyping) algorithms. We also performed mutation analysis of BRAF (codon 600) and NRAS (codons 12 and 61) in tumor samples.

Results: A missense mutation (p.D84Y; c.250G>T) was found in exon 2 of the CDKN2A gene. Segregation analysis of the variant was compatible with an association with the disease, since it was present in four affected family members. This mutation was predicted to affect protein function using SIFT and PolyPhen analysis. LOH analysis revealed one tumor with monoallelic loss and another with biallelic loss of the CDKN2A locus. We did not find BRAF and NRAS mutations in the tumors.
Conclusions: We show evidence that the p.D84Y missense mutation predisposes to melanoma since it is localized in a conserved domain and co-segregates with disease in this pedigree. These findings may have important implications for genetic counseling, molecular testing, and clinical management of Portuguese melanoma-prone families.

7012 POSTER

Photodynamic diagnostics of skin and mucosal lesions

J. Liutkeviciute Navickiene, L. Plesniene, A. Mordas, L. Rutkovskiene.
Vilnius University Oncology Institute, Laboratory of Laser and Photodynamic Treatment, Vilnius, Lithuania

Background: Porphyrin-enriched tumor tissue irradiation with fluorescence excitation system leads to emission of pink-red fluorescence. This principle is used as a diagnostic procedure and is called photodynamic diagnosis (PDD). The aim of this work was to investigate the possibilities of PDD in skin and mucosal lesions diagnostics.
Material and Methods: Photodynamic diagnostics measurements were performed in 68 patients with malignant, premalignant and benign skin and mucosal lesions for detection of the foci of squamous cell carcinoma, basal cell carcinoma, primary and metastatic adenocarcinoma, chondrosarcoma. Two different photosensitizers have been used – intravenous injection of hematoporphyrin derivate (HpD) and the topical application of 5-aminolevulinic acid (ALA) induced protoporphyrin IX (PpIX). We used the simple and friendly fluorescence excitation system based on blue light emitting diodes. For the patients with advanced malignant disease, HpD was injected i.v. and 12−24 hours after the injection, malignant lesions were illuminated with violet-blue (405 nm) light for cancerous tissue detection. PDD for patients with T1−2 was carried on 3−6 hours after topical ALA application. The evaluated fluorescence data was correlated with cytological and/or histopathological tissue examination data.
Results: Red or red-pink fluorescence was observed in 72 malignant epithelial tumors; 43 of them fluorescent sharp, 27 – not so intensive. 2 malignant tumors – nasopharyngeal area chondrosarcomas – had no fluorescence. The most intensive red fluorescence was detected in thin superficial malignant lesions. From 165 benign lesions, very slight fluorescence has been detected in a few haemangiomas, paratracheal papillomas, one fragment of herpes zoster and some superficial open wounds with very intensive capillarity. Fluorescence of these benign lesions was different from malignant – not so red, bluer and less intensive. Nevi, papillomas, keratosis, scars and foci of psoriasis had no fluorescention.
Conclusions: Photodynamic diagnostics can be used for complete visualization of malignant lesions after the topical or systemic application of a tumour selective photosensitizer. It has been shown to be high effective in malignant superficial skin and mucosal lesions diagnostics. PDD may be required to optimise the detection of lesions in the post-PDT patients. Fluorescence detection following i.v. injection of HpD or topical application of ALA provides no difference.

7013 POSTER

Multicenter phase II study of chemo-immunotherapy in the treatment of metastatic melanoma

F. Recchia[1], G. Candeloro[1], S. Necozione[2], L. Fumagalli[3], S. Rea[4].
[1]Ospedale Civile, Oncologia, Avezzano, Italy; [2]Università degli Studi de L'Aquila, Epidemiologia Clinica, L'Aquila, Italy; [3]Ospedale San Gerado, Unità Operativa di Chirurgia Generale 3, Monza, Italy; [4]Fondazione "Carlo Ferri", Chirurgia Oncologica, Monterotondo, Italy

Background: Combining chemotherapy and immunotherapeutic agents such as interleukin-2 and interferon alfa-2b may improve treatment results in metastatic melanoma [MM] patients compared with chemotherapy alone. This prospective study evaluated the potential efficacy of a bio-chemotherapy regimen followed by maintenance biotherapy for the treatment of MM.
Materials and Methods: Twenty-two patients with stage IV melanoma were treated for 5 consecutive days with cisplatin 20 mg/m^2, vinblastine 1.6 mg/m^2, and dacarbazine 160 mg/m^2 followed by pegylated ?-interferon 2b 50 ?g every week, subcutaneous interleukin-2 [IL-2] 1.8 M I.U. and oral 13-cis retinoic acid [13-cis-RA] 0.5 mg/kg, both given 5 days/week for 3 weeks each month. To eradicate minimal residual disease, maintenance biotherapy was continued in patients who achieved clinical benefit after 6 courses of bio-chemotherapy. The primary endpoint was response; secondary end-points were the evaluation of the immunological parameters, toxicity, progression-free survival [PFS], and overall survival [OS].
Results: Twelve patients [54.5%] achieved a response, and 7 [31.8%] maintained stable disease for at least 6 months on maintenance biotherapy. The median PFS and OS were 23.3 months and 45.7 months, respectively. The most important toxicities from chemotherapy were grade 3 and 4 neutropenia and thrombocytopenia in 41% and 18% of patients, respectively, while grade 2 autoimmune reactions were observed in 21% of patients after maintenance biotherapy. A prolonged enhancement of immunological function was observed in the 19 patients treated with maintenance therapy.
Conclusions: Six cycles of bio-chemotherapy followed by maintenance immunotherapy is well tolerated and shows significant activity in patients with MM.

7014 POSTER

Thin (<1 mm) and in-situ melanomas during 1989–2004 in Helsinki, Finland – clinical outcomes and prognostic factors

P. Stahlberg[1], S. Virolainen[2], O. Saksela[3], T. Jahkola[1]. *[1]Helsinki University Central Hospital, Department of Plastic Surgery, HUS Helsinki, Finland; [2]Helsinki University Central Hospital, Department of Pathology, HUS Helsinki, Finland; [3]Helsinki University Central Hospital, Department of Dermatology, HUS Helsinki, Finland*

Background: The prognostic factors associated with poor outcome in thin melanomas are not well known. Our purpose was to study the ratio and long term prognosis of thin melanomas in Helsinki region and to find out whether the tumor or patient characteristics that were studied were related with poor outcome.
Materials and Methods: The slides of Breslow thickness <1 mm, in-situ melanomas or lentigo malignae were reviewed, n = 301. The patient registries and the Finnish population registry were studied for the follow-up data.
Results: The mean age of the patients was 54.3 years, 58% were women. The mean follow-up was 6.7 years. There were 246 invasive cases (82%). 60.1% of all cases (n = 301) were of Clark level III, 18.6% were of Clark level II and 3.0% were of Clark level IV. Ten recurrent cases (4.1%) were invasive melanomas (<1 mm), three of which (1.2%) had died of melanoma. Four patients (1.6%) were alive with local recurrence, one with nodal recurrence and one with distant metastasis. One patient had died of coronary disease and was post-mortem diagnosed with metastatic melanoma. The group of in-situ melanomas and lentigo malignae (n = 55) contained one recurrent case with rapid nodal metastasis. The patient was diagnosed with nodal metastasis 13 days after the melanoma diagnosis, and had died of melanoma with distant metastasis one year later. The histological factors that were studied (Breslow thickness, tumor pigment, ulceration, solar elastosis and tumor-infiltrating lymphocytes) did not predict recurrence. The amount of pigment predicted overall survival, but tumor ulceration, the amount of lymphocytes and solar elastosis did not.
Conclusions: The treatment results in invasive melanomas (n = 246) were slightly better than expected (the recurrence rate was 4.1%). Pigment content of the tumor was the only prognostic factor for overall survival of the histological factors studied (pigment, ulceration, solar elastosis and tumor-infiltrating lymphocytes). These histological factors were not associated with disease recurrence. New prognostic factors are needed for staging thin melanomas.

7015 POSTER

The interest of raw data in dynamic contrast-enhanced ultrasonography (DCE-US) for the quantification of perfusion changes in in-transit melanoma metastasis (MM) treated by high doses of chemotherapy: does it predict the response?

L. Chami[1], A. Cavalcanti[2], S. Bidault[1], B. Benatsou[1], S. Koscielny[3], P. Peronneau[1], S. Bonvalot[2], N. Lassau[1]. *[1]Institut Gustave Roussy, Departement d'Imagerie, Villejuif, France; [2]Institut Gustave Roussy, Departement de Médecine, Villejuif, France; [3]Institut Gustave Roussy, Departement de Biostatistique, Villejuif, France*

Background: To evaluate the performances of a new method of quantification of perfusion in DCE-US using the raw data for the prediction of tumoral response.
Materials and Methods: In this prospective study patients suffering from localised MM were included from march 2004. All of them were treated by high doses of chemotherapy (Melphalan +/− TNFa) delivered under isolated limb perfusion. B mode morphological imaging followed by functional examination using contrast agent injection (SonoVue, Bracco, Italy) and a perfusion and quantification software (VRI and CHI-Q, Toshiba, Japan) were performed before treatment and at D+1, D+7 and D+90. Contrast