FROM DYSPLASTIC NEVUS TO MELANOMA: FUNCTIONAL PROTEOMIC APPROACH FOR THE IDENTIFICATION OF BIOMARKERS

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Objective

The project ultimately aims to identify biomarkers from serum or other biological fluids helpful for early diagnosis of melanoma. Proteomic analysis combined with advanced skin imaging technology, such as confocal microscopy, is directed to the identification of different types of benign melanocytic lesions, as well as to the characterization of different melanomas and dysplastic nevi, in order to understand different tumour progression behaviours and to identify possible melanoma precursors.

The first year of the study has been primarily dedicated to the recruitment of patients and matched healthy donors, to the set up of protocols for the recover of proteins from interstitial fluids and serum and to the development of standardized protocol for serum protein analysis by means of nanoparticles, for the identification of serum biomarkers and their validation, and to the characterization of morphological aspects of melanocytic tumours as evaluated by means of *in vivo* confocal microscopy.

– *Recording of clinical and instrumental information, and collection of biological samples from the study population.*
  279 melanocytic skin lesions from 256 patients were studied in the project. Each lesion was examined by means of methods for advanced skin imaging.

– *Lesion imaging.*
  - Digital dermoscopic image acquisition (Fotofinder Teachscreen), employing 20 fold to 50 fold magnifications, of the whole leson and significant details.
  - Since May 2008, digital dermoscpic images were also acquired using polarized light dermoscopy DermLite-Photo (3Gen).
  - *In vivo* confocal image acquisition of each studied lesion, obtaining images from three different depth levels (superficial epidermis, dermal-epidermal junction and superficial dermis).

– *Collection and storage of serum samples for proteomic analysis.*
  Based on results from dermoscopic and *in vivo* confocal image acquisition, 90 patients have been recruitd starting November 2007, matched with 30 healthy donors for further assessment. Serum collection was performed according to the protocol previously established by the participants to the oncoproteomic network.
  For each patient recruited, three aliquots of serum were stored at – 80°C and shipped to Lance Liotta laboratory at GMU, where dr. Caterina Longo has been collaborating, since Jenuary 2008, to the set up and standardization of the procedure for the appropriate application of nanopartricles in proteomic analysis.

– *Collection and storage of TIF samples for functional proteomics.*
  The interstitial fluid that perfuses the tumor microenvironment, a biological fluid representing a novel and highly promising source of biomarkers for cancer, was collected from freshly dissected lesions, then the protein mixture was recovered and stored for further analysis.

– *Preparation, updating and management of the population database.*
  - Sensible data coding, image storage and privacy protection.
  - Image transfer on dedicated Server, for data security.
  - Storage of information in MS Excell format and update of the information with histologic report for each case.
  - Data management and organization and maintenance of the database.

– *Image description.*
  Development of MS Access platform for the evaluation of dermoscopic and confocal microscopic aspects of acquired lesions, based on literature research. Export of data from MS Access to STATA and SPSS formats for statistical analysis.

Statistical analysis and results

– Study of the inter- and intra-observer reproducibility of the parameters employed for the description of confocal images from melanocytic lesions.
– Study of characteristic aspects in different melanocytic lesion subgroups, by means of dermoscopic, confocal microscopic and histologic evaluation, for the identification of different subgroups within benign and malignant lesions aiming to a better correlation between morphological and proteomic parameters.
  - Identification of characteristic parameters in Spitz nevi by means of confocal microscopy. The study aimed to the histopathologic correlation of confocal microscopic features and to the differential diagnosis between Spitz nevi and melanoma and Clark nevi.
  - Classification of common nevi, based on the identification of characteristic confocal microscopic aspects and biological behaviour.
  - Identification of characteristic aspects of dysplastic nevi by means of confocal microscopy, and histopathologic correlates.
  - Identification of characteristic features in lentigo maligna type melanoma and nodular melanoma by means of confocal microscopy, to refine the classification of melanomas based on *in vivo* morphological aspects.
– Set up of a protocol to recover proteins from TIFs.
  - Interstitial fluids that perfuses the tumor microenvironment were collected from freshly dissected lesions. In order to evaluate the critical steps of the process necessary to preserve and enrich proteins for proteomic analysis, several precipitation and/or purification protocols were evaluated. A mixture of acidic acetone and methanol as precipitating agent was selected as routine protocol, based on the amount of proteins recovered and best 2-DE resolution.
  - The protein mixture recovered from the fluid was then analyzed by either 1-dimensional or 2-dimensional polyacrylamide gel electrophoresis in combination with

western blotting. This approach might provide a picture of the protein components of TIFs in melanoma.

- As expected, TIFs contained several serum proteins, as judged by comparison with the published catalogs of normal human serum proteins, but contained also several phosphoproteins recognized by an antibody directed against putative substrates of proteinkinase B/Akt and of protein kinase C. The proteins recognized have been excised and will be subjected to identification through Maldi-Tof analysis.

Activities in collaboration with the George Mason University, WA, USA

- Development and standardization of the procedure for the appropriate application of nanopartricles in proteomic analysis.
- Comparison of the capability of nanoparticles in the detection of small and transient proteic molecules, such as PDGF, in respect of routine methods, such as SDS PAGE Electrophoresis, ELISA and Mass Spectroscopy.
- Development of standardized protocol for serum protein analysis by means of nanoparticles, for the identification of serum biomarkers and their validation.

Publications of the project

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Pellacani G, Scope A, Ferrari B, Pupelli G, Bassoli S, Longo C, Cesinaro AM, Argenziano G, Hofmann-Wellenhof R, Malvehy J, Marghoob AA, Puig S, Seidenari S, Kittler H, Soyer HP, Zalaudek I. New insights into nevogenesis: *in vivo* follow up of melanocytic nevi by reflectance confocal microscopy. Manuscript in preparation.

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Segura S, Pellacani G, Puig S, Longo C, Bassoli S, Guitera P, Palou J, Menzies S, Seidenari S, Malvehy J. *In vivo* microscopic features of nodular melanomas: dermoscopy, confocal microscopy and histopathologic correlates. *Arch Dermatol* (in press).