ImmunoTools *special* Award 2015

Francesco Facchiano, MD.
Senior Researcher, Group Leader

Dept. of Hematology, Oncology and Molecular Medicine,
Istituto Superiore di Sanità, 00161 Rome, Italy

Serumproteomics of cancer using *in vitro* and *in vivo* models to develop new diagnostic-therapeutic strategies

Cutaneous melanoma is the most malignant skin cancer and its frequency is rapidly growing in the world. When diagnosis is late, prognosis is poor. Therefore, the identification of new biomarkers useful for early diagnosis or new therapeutic approaches is a very important issue. The most simple and informative biological sample is the serum, due to its easy collection and storage, but analysis of serum proteome is actually very difficult due to the presence of abundant and large proteins, e.g. albumin and immunoglobulins. To overcome this problem, depletion techniques are frequently applied to remove such abundant proteins that are cargos whose function is to transport smaller and less abundant signals. The removal of these larger proteins may lead to the waste of smaller molecules carried by the cargos. This is one of the reasons to explain why many efforts spent in serum proteomics analyses during the last decades did not generate significant results in new biomarkers discovery. To address this crucial question, we recently developed a new pre-prep technique to improve the accessibility and solubility of serum proteins without removal of any cargo protein. This technique, called TRIDENT (Three DENaturation Treatments) (Verdoliva 2013) consists of application of 3 different denaturation protocols before to fractionate the whole serum (or other complex samples like cell extracts) by SDS-PAGE and proteins identification by mass spectrometry. The comparison of proteins identified by the different denaturation protocols reveals a large amount of information mainly related to protein posttranslational modifications, due to their higher or lower sensitivity to denaturation. Our first studies applied TRIDENT technology to sera from melanoma patients and healthy subjects allowing to identify a number of serum proteins never before involved in melanoma diagnosis or pathogenesis. Further, the identified patterns suggested some new biochemical pathways potentially involved in early stages of melanoma development and progression. New studies were then carried out by TRIDENT analyses to characterize protein extracts from 10 different human cell lines (8 melanoma and 2 controls, i.e. melanocytes and keratinocytes cell lines) indicating some

signals secreted by melanoma cells possibly involved in melanoma aggressiveness and metastatic dissemination. Interestingly, we found some functional features in serum melanoma proteomes compared to healthy controls very similar to some biochemical pathways found in cell extracts studies comparing melanoma cells with higher or lower aggressiveness and control cells. In particular, some proteolytic, angiogenetic and signalling molecules were found as the most interesting. Therefore a larger study involving human melanoma stem-like cells (melanospheres) and a murine model of melanoma have been planned to investigate in deep the involvement and these mechanisms. The following signalling molecules have been selected among the large collection of ImmunoTools reagents, to confirm the observed findings: rhFGF-a/FGF-1, rhFGF-b/FGF-2, rhGalectin-1, rhGalectin-3, rhG-CSF, rhGM-CSF, rhHGF, rhIFNa1b, rhIFNa2a, rhIFNgamma, rhIL-1ra, rhIL-6, rhIL-7, rhIL-17A, rhIL-17B, rhIP-10/CXCL10, rhMCP1/CCL2, rhMCP2/CCL8, rhPDGF-AA, rhPDGF-BB, rhRANTES/CCL5, rhTNFα, rhVEGF-A/VEGF-165.

These recombinant molecules will be tested in cellular experiments (proliferation and invasion assays), alone or in combination, to confirm their role in human melanoma aggressiveness. The most involved pathways will be then tested in a murine metastatic melanoma model (the protocol to perform these studies was already submitted to the Ethical Committee and approved). Since these signalling molecules may be also measured in serum by means of ELISA assays, they will be also evaluated as novel predictive indicators useful as diagnostic or prognostic markers of melanoma.

Reference:

Verdoliva V, *et al.* Differential denaturation of serum proteome reveals a significant amount of hidden information in complex mixtures of proteins. PLoS One. 2013;8(3):e57104. doi: 10.1371/journal.pone.0057104