ImmunoTools *special* Award 2018

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New and improved methods for characterization of tumour-specific markers in exosomes

Extracellular vesicles (EVs) are small vesicles released by cells, which depending on their biogenesis are classified as exosomes, microvesicles or apoptotic bodies. EVs provide an invaluable tool for the analysis of the physiological processes occurring in an individual because they transport, in biological fluids, biomolecules secreted from diverse tissues. Furthermore, EVs have been shown to play important roles in intercellular communication, in health and diseases such as cancer and have been shown to be involved in processes such as metastasis or tumour immune evasion. The detection of biomarkers present in EVs requires techniques with a high sensitivity. However, the lack of widespread and affordable methodologies for high throughput EV analyses is a barrier for studies on the importance of these biomarkers in large patient cohorts.

With the aim of developing such tools for EV analysis, we have evaluated the critical parameters necessary to optimise several immunocapture assays. Comparison of the different techniques: Western blot, ELISA, flow cytometry and lateral flow, demonstrates that the use of different combination of tetraspanins and tumour markers antibodies can result in very different outcomes when used in the different techniques. In fact, the optimal combinations and concentrations of antibodies for use in each technique had to be optimised separately. These results are the consequence of translating methods originally established for detection of soluble molecules into the detection of molecules in vesicles. This is a key question when optimizing new techniques for the detection of tumour markers in exosomes of biological samples.

Our objective is to further optimize the available techniques in order to detect possible exosomal tumour associated markers directly in patients' biological fluids and generate high-throughput tools that facilitate investigation of the role of the presence of these markers in tumour derived exosomes. To address this question we will take into account the biochemical and bio-physical properties of exosomes in biological fluids for the optimization of the available immunocapture techniques. Additionally, we will validate

these techniques for several possible exosomal tumor associated markers in exosomes from patient samples. Moreover, with flow cytometry we can optimize a multiplex assay for the detection of several proteins or markers in the same assay. The ImmunoTools Award reagents include a wide variety of antibodies against tumour associated proteins which have been already detected in exosomes and have been proposed as candidates for exosomal tumour associated markers. A set of these antibodies can be used for the validation of the proposed techniques as well as for the validation of exosome biomarkers in cancer patient samples. This work will provide new optimized widespread and cost-effective methodologies such as flow cytometry for high throughput EV analyses, an essential tool to validate biomarkers in large patient cohorts. These technologies can be easily translated to the clinics as a tool for liquid biopsy, which, with the appropriate validated biomarkers, could allow in the future early diagnosis, prognosis, selection of a therapy or treatment monitoring of a cancer patient in a non-invasive way.

ImmunoTools *special* Award for **Carmen Campos Silva** includes 22 reagents

FITC- conjugated anti-human CD9, CD63, CD147, CD24, CD105, CD55, CD59, HLA-II, Annexin V, Control-IgG, Control-IgG2a

PE- conjugated anti-human CD9, CD63, CD147, CD24, CD105, CD55, CD59, HLA –ABC, CD66e (CEACAM5), Annexin V, Control-IgG, Control-IgG2a

DETAILS more AWARDS