Super-resolution profilling of proteomic derived colorectal cancer metastasis markers

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Colorectal cancer (CRC) is a major health problem in developed countries(1). CRC incidence is the first in cancer considering both genders simultaneously, and the second major cause of cancer-associated death in developed countries. Prospects support that the occurrence of the malignancy will increase around 2.6% per year in the upcoming years(2). The high mortality ratio of this malignancy is largely due to the fact that patients are diagnosed in late stages of the disease where metastatic lesions have already been produced. To gain further insights into underlying mechanisms related to colorectal cancer metastasis, our research group has been performing proteomic studies using isogenic cell lines, which share the same genetic background but differ in their CRC metastatic behaviour. Recently, to study CRC metastasis to liver, we have performed spatial proteomics analyses using the CRC pair of isogenic cells with high metastatic ability to liver (KM12SM) and low or non-metastatic ability (KM12C cells). One of the most altered properties in CRC metastasis to liver consisted of membrane trafficking related markers (Figure 1). More specifically, proteins of the AP-type membrane coat adaptor complex and COPI vesicle coat proteins were found to be highly upregulated in several subcellular localizations in metastatic cell lines compared with the non-metastatic cell lines.

To study the functional implications of these alterations, we have employed Stimulated Emission Depletion (STED) nanoscopy (3) (Figure 2) to detect subtle changes in the localization and distribution of the altered proteins. In STED nanoscopy, the principle of stimulated emission is exploited to alter the shape of the effective point-spread function and achieve an increase in resolution. To maintain the expression levels of the proteins at the same extent to those detected in the proteomic studies, we have opted for CRISPR/Cas9 gene edition to tag the altered proteins. Among the different available tags, we chose the SNAP-tag as it would allow for labelling with small organic dyes that are more photostable and hence more suited for STED nanoscopy. Furthermore, it also provided us the freedom to change the used fluorescent marker when desired without having to edit again the cell lines. From the altered markers, we focused on proteins involved in the retrograde vesicle trafficking from the extracellular medium and towards the inside of the cell, to try to identify novel proteins associated to the vesicle trafficking and related to CRC metastasis, and to get further insights into mechanisms involving retrograde vesicle formation. To complement the superresolution microscopy studies and probe the interactions established by the altered proteins we made use of lifetime-based FRET determination to be able to follow these interactions as they happen in living cells.

We expect the information obtained from these studies to shed light into the mechanisms underlying CRC metastasis and to pinpoint possible therapeutic targets. Furthermore, the proteomic characterization from other tropisms in CRC metastasis might ultimately lead to the discovery of a general diagnosis panel for all CRC metastasis and even for common treatment strategies.

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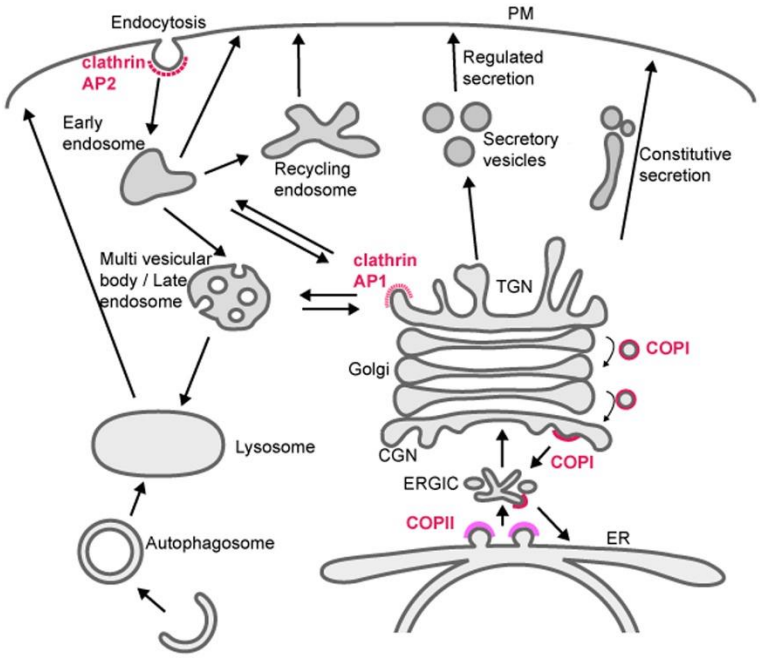

Figure 1: Schematic of vesicle transport inside the cell. The different adaptor proteins involved in several ways of anterograde and retrogtade transport are also shown. Adapted from (4)

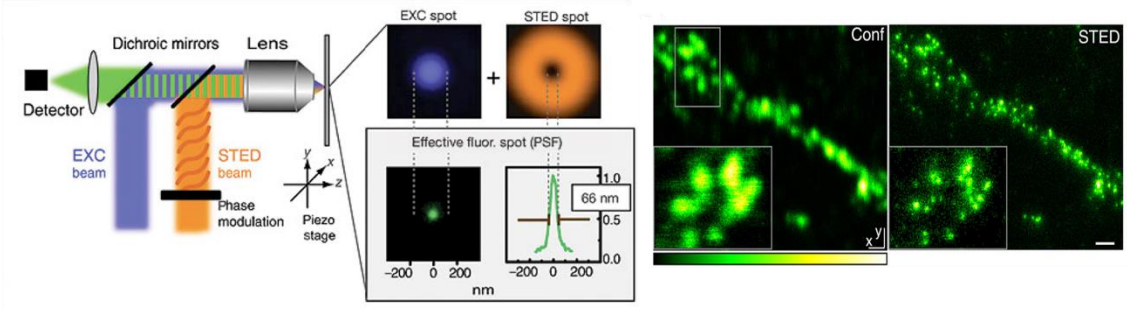

Figure 2: STED principle and application compared with confocal scanning microscopy. Adapted from (3).

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