Macrophages are involved in almost every disease. They are recruited during inflammation to exert their inflammatory and phagocytosis functions. Tissue-resident macrophages are not a homogeneous population. In fact, they present different phenotypes which can be influenced by their environment. Macrophages activation is often represented in two ways: M1 pro-inflammatory and M2 anti-inflammatory polarization. This description is over-simplified because macrophages activation depends on many parameters such as their origin, environment etc. During tumorigenesis, macrophages are recruited to the tumor site to become tumour associated macrophages (TAMs). However, the tumor creates an immune suppressive environment, which orients TAMs toward the anti-inflammatory M2 phenotype. One challenge is to counteract this local immune suppression observed in numerous cancers. More and more therapies are being developed in order to find a way to control macrophages' phenotype switching in order to boost the immune response in cancer patients. Pro-inflammatory macrophages can be activated by several stimuli triggering signaling cascades. As stimuli, we can find Toll-like receptors (TLR) ligands (LPS, CpG-ODN, Taxol . . . ). TLR-based therapies could be thus very interesting.

Our research goes in that direction. In fact, we work on an enzyme called proprotein convertase 1/3 (PC1/3). PC1/3 was first associated with the neuroendocrine system. More recent studies have demonstrated that this enzyme is also expressed in immune cells like macrophages and lymphocytes. In PC1/3 knockout mice, a massive cytokine response was registered following the stimulation of TLR4 [1]. Another study confirmed the role of PC1/3 in cytokines release in rat alveolar NR8383 macrophages [2]. In the present study, we want to know the impact of PC1/3 knockdown (KD) on NR8383 macrophages activation after TLR4 (LPS) and TLR9 (CpG-ODN) stimulations. We used a complete proteomic approach to answer this question. In the first part, a shotgun experiment was performed on the macrophages supernatants stimulated with LPS from 1 h to 72 h. More than 1400 proteins were identified and 18 proteins were specific to PC1/3 inhibition. Some of them are involved in Th1-cell activation and inflammatory response. We also identified immune factors such as danger signals, cytokines and chemokines which are secreted by PC1/3 KD macrophages earlier compared to non-target shRNA (NT) macrophages [3]. We then focused on the secretion of chemokines and cytokines by cytokines arrays and confirmed the fact that PC1/3 KD macrophages secrete more pro-inflammatory factors such as IL6, TNF-α, CXCL10, IL-1α and β. After LPS treatment, the secretion of some of these factors was enhanced. Interestingly, the stimulation with
CpG-ODN triggered a slightly different secretion than the one observed under LPS. In fact, PC1/3 KD macrophages secrete more CXCL2 and IL1-α. One important observation was that PC1/3 KD macrophages secretomes, obtained after LPS stimulation, have an anti-tumor activity on breast (SKBR3) and ovarian (SKOV3) cancer cells, meaning that these macrophages secrete killing factors [3]. To understand why such secretion is observed in PC1/3 KD macrophages, we studied the intracellular proteins by mass spectrometry. The first finding from this proteomic data was the big impact of PC1/3 knockdown on the cytoskeleton. Co-expression network analysis showed that actin-related proteins and exosomal proteins were overexpressed in PC1/3 KD macrophages. A complete reorganization of the cytoskeleton was highlighted with the development of a lot of filopodia in PC1/3 KD cells leading us to affirm that macrophages are more active when PC1/3 is inhibited. This cytoskeleton reorganization has a huge impact on the formation of multivesicular bodies (MVB) and the trafficking of some proteins such as the TLR9 receptor [4]. In fact, immunofluorescence studies showed that TLR9 trafficking is modulated in PC1/3 KD macrophages and is aggregated within MVB. The higher number of MVB and exosomes can be one explanation for the cytokines release observed in PC1/3 KD macrophages. Moreover, calcium homeostasis is also modulated as a consequence of cytoskeleton rearrangement. Basal calcium concentration is higher in PC1/3 KD macrophages compared to NT macrophages and increases following LPS stimulation. Calcium and cytokines release are often linked. Furthermore, calcium can also influenced the TLR signaling pathway. We investigated TLR9 and TLR4 signaling pathways by studying the level of degradation of IκB-α after LPS or CpG-ODN stimulation. We noticed that the NF-κB transcription factor is enhanced in both cases in PC1/3 KD macrophages. By proteomic, we also demonstrated that the two pro-inflammatory transcription factors STAT1 and STAT2 are over-expressed whereas the level of phosphorylation of STAT3, which is known to inhibit NF-κB signaling, is lower in PC1/3 KD macrophages. This last point could be interesting because STAT3 is a known target in immunotherapy of cancer.

In this study, we demonstrated, thanks to proteomic, that PC1/3 KD macrophages present all the characteristic of activated pro-inflammatory macrophages. We are able to enhance TLR4 and TLR9 signaling pathways leading to the secretion of pro-inflammatory factors and anti-tumor factors (Fig. 1). We can control their activation by controlling one enzyme, PC1/3. In this perspective, it should be interesting to verify our concept on other TLR. In a tumor context, PC1/3 inhibition in macrophages may reactivate them and lead to a cytokine storm after stimulation “at distance” with a TLR ligand. Therefore, we name these PC1/3 inhibited macrophages the “drone macrophages”. They constitute an innovative cell therapy to treat efficiently tumors.

**Conflict of interest**

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.euprot.2016.03.003.

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