1. Did PDL-1 content increase in cultured cancer cells contribute to CTL-induced lysis in in vitro co-culture experiments monitoring Cr release, under hypoxia conditions?

From the studies I have presented using coculture systems, the link between hypoxia, PDL1 expression "by tumor cells" and resistance to CTL has not been clearly established. First because in the specific settings and models we have looked hypoxia effects on cancer cells, PD-L1 remains poorly expressed (in the absence of added IFNg). Second, because most of the immune effector cells used in the coculture assays (CTLs, NK) have limited amount or no expression of PD-1 at their surface.

Nevertheless, in various cellular/mouse models of cancer cells more less Epithelial or Mesenchymal, It was shown that increased PD-L1 production by the cancer cells (in cells with more mesenchymal phenotype) contributes to immune suppression and resistance to lymphocyte-mediated lysis However in this case, the link with hypoxia has not yet to be established.

2. Congratulation, very nice talk. I have 3 questions:
    a. In the model that you have used, I have seen that most of them generate cold tumors (B16, CT26 and 4T1). When you treat mice with HDC, do you have an increase of proteasome activity in cancer cells? Do you think that the tumor shrinkage observed is due to the increase of the level MHCI levels that is needed to present intracellular antigens degraded by proteasome?

Very good question. These experiments were performed ten years ago and focusing on autophagy deregulation. I am really not sure that variations in MHC expression were investigated in the different treatment conditions. But considering links between autophagy and proteasome, also deregulation of p62 in these systems, it would be interesting to look at.
https://pubmed.ncbi.nlm.nih.gov/21810913/

    b. I was wondering if in your model Hypoxia may regulate CD4 subtypes as TH1 and Eomes?

We have not looked. clearly the potential role of hypoxia on CD4 populations and in general on T cells Is something that deserves further investigations due to controversial results obtained in the literature, depending models, contexts (mouse/human), and differentiation states of the T populations.

    c. In hypoxia condition are cancer cells more sensitive to IFN-gamma that induce an up-regulation of PD-L1?"

In the Noman et al. study, IFNG was used as a control for PD-L1 induction on MDSCs and also on tumor models https://pubmed.ncbi.nlm.nih.gov/24778419/ . In general, IFNg is very potent to induce PD-L1 expression especially in normoxic cells, even at low doses. Therefore, I don't expect hypoxic cells are "more" sensitive to IFNg-induced PD-L1. However, this question should be further investigated in various mouse and human models.

3. Could miR-210 be secreted and participate to a dialog between tumor cells and their microenvironment?

Good question. It the initial study only the intratumor miR-210 was investigated.

https://pubmed.ncbi.nlm.nih.gov/22962263/

More recent studies from our lab and collaborators in Luxembourg have shown a role for extracellular miRs in cell cell communication between cancer cell – immune effectors such as NK cells. miR210 and another miR, miR23a, can be transferred via tumor-derived vesicles and play a role. In this context, miR23a was particularly potent. Please have a look to this reference for more details.

https://pubmed.ncbi.nlm.nih.gov/27141372/