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Topic: SIGNALING AND EPIGENETICS IN CANCER

Dissecting the role of Oncostatin M (OSM) signalling in the hepatic tumour-preceding inflammation and fibrosis

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Hepatocellular carcinoma (HCC) is one of the deadliest cancers and the most common liver tumour. The mechanisms driving HCC progression are very complex and, usually, other liver diseases precede HCC hindering HCC early diagnosis. Indeed, unravelling the mechanistic insights of HCC onset may help to discover novel molecular targets of clinical interest. Among others, chronification of inflammatory-fibrotic loops triggers liver damage and cirrhosis, which usually precede HCC. These cross-regulatory loops promote tissue remodelling as well as the recruitment and/or activation of non-parenchymal liver cells, mainly hepatic stellate cells (HSCs) and resident or infiltrated immune cells.

In this context, the inflammatory cytokine Oncostatin M (OSM) signalling may be a promising target. OSM is a cytokine from the Interleukin-6 family that, acting through its receptor OSMR, promotes tumorigenesis, tumour microenvironment remodelling and metastasis in different cancer types (e.g. breast or pancreatic cancer). Moreover, OSM/OSMR pathway plays relevant roles in liver development, regeneration and fibrosis, although little is known about its function in HCC onset and progression.

Thus, we hypothesised that OSM signalling may contribute to HCC onset and progression by affecting, not only tumour cells, but also immune and stromal populations in the pre-tumour and tumour microenvironment.

To test our hypothesis, we studied HCC formation in mice lacking OSM signalling (*Osmr*-KO model) compared to controls (*Osmr*-WT). We chemically induced HCC by using two models with different inflammatory status: low-inflammation Diethylnitrosamine (DEN)-induced model and high-inflammation DEN combined with carbon tetrachloride (DEN+CCl_4) model. Moreover, DEN+CCl_4 model was studied at two different time points of HCC progression. We characterized liver damage and tumour development as well as the dynamics of populations from the tumour microenvironment by flow cytometry and single nuclei RNAseq (snRNAseq), among others. We complemented these results with *in vitro* experiments of HSCs stimulated with OSM and data obtained from two independent cohorts of patients and publicly available databases.

First, in our long-term HCC models, we discovered that the lack of *Osmr* decreased tumour number and size in the DEN+CCl_4 pro-inflammatory model, while it did not affect inflammation-independent tumorigenesis. Moreover, *Osmr* depletion alleviated liver inflammation and prevented HCC-associated liver damage. In early stages of the DEN+CCl_4 model, we observed that *Osmr* depletion promoted the remodelling of the immune compartment in the pre-TME, according to our results obtained by flow cytometry and snRNAseq. In general terms, in *Osmr*-KO mice, we detected an increase in immune populations associated to anti-tumour activity. Interestingly, we also found that *Osmr* was mainly expressed by HSCs. When stimulated with OSM, LX2 cells (an *in vitro* model of HSCs) upregulated myofibroblast-associated markers such as *FAP*, *LOX* or *PLOD2*, as well as inflammatory cytokines (*LIF* or *IL6*). In line with these results, in HCC patients' data, *OSM* and *OSMR* expression positively correlate with markers of fibrosis and inflammation as well as with the infiltration level of cancer-associated fibroblasts. Finally, we confirmed that *OSM* and *OSMR* are overexpressed in cirrhotic/pre-HCC samples, as well as high expression of

OSMR is associated with decreased survival in patients' cohorts.

Our results strongly support that OSM pathway is a key regulator of HCC-promoting inflammation-fibrosis crossregulation. We have demonstrated that immune-derived OSM activates OSMR-expressing HSCs to drive inflammatory and fibrotic loops that prompt HCC progression. Thus, OSM/OSMR pathway may have a potential therapeutic role as target upon chronic liver damage to avoid tumour development.