REPORT
25th SOCIAL RETURN OF THE RESEARCH
CANCER

FATTY ACIDS IN THE DIET AND METASTASIS: IDENTIFICATION OF NEW THERAPEUTIC STRATEGIES AGAINST METASTASIS-INITIATING CELLS

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1. Results obtained

In this project we proposed to study the mechanism of action of metastasis-initiating cells. Previous studies from our laboratory demonstrated that these cells depend on fat metabolism to exert their prometastatic function. We proposed two overarching objectives: What are the transcriptional changes that prometastatic fatty acids cause specifically in metastatic stem cells and the metastatic niche? And what are the consequences of the long-term prometastatic epigenetic memory of metastasis-initiating cells in tumor development? Can these mechanisms and changes be prevented or reversed?

Thanks to this project, we have identified the main fatty acids in our diet that activate metastatic cells, such as palmitic acid. We have discovered that exposing the tumor to high levels of this fatty acid establishes an epigenetic memory. That is, once tumor cells have been stimulated with palmitic acid, they become much more sensitive to this fatty acid, which very significantly increases their metastatic potential. We have discovered the precise epigenetic mechanism that establishes and maintains this memory, through post-transcriptional modifications in specific histones. Furthermore, we have identified the protein responsible for this epigenetic modification, which is allowing us to develop new therapies to erase this prometastatic memory.

Finally, we have identified that tumor neuronal and glial cells play an essential role in its metastatic progression. We have studied the glial populations (i.e. Schwann cells) that are associated with the tumor, and signaling pathways that activate their prometastatic activity. One of these pathways promotes the secretion of a specific extracellular matrix that allows metastatic cells to migrate out of the tumor and invade other organs. We have shown that inhibition of the secretion of this extracellular matrix prevents metastatic tumor progression. These studies have been carried out in oral carcinomas and melanoma.

Likewise, we have studied the immune stroma that is generated specifically in metastatic lesions. Through cell-to-cell transcriptome studies (single cell RNA-sequencing), and metabolome and lipidome studies, we have identified that certain populations of neutrophils and macrophages have metabolic reprogramming that promotes the immunosuppressive state of metastases. We are carrying out functional

trials to verify whether by inhibiting this metabolic state associated with metastasis we are able to modulate the metastatic potential of the tumor.

Furthermore, we have identified the palmitoylome of metastatic cells and one of the proteins involved in said palmitoylation, the DHHC14 protein. We are in the process of developing drugs that inhibit this protein and studying its antimetastatic therapeutic potential.

2. Relevance and possible future implications

With this project we have identified a prometastatic epigenetic memory induced by certain very common fatty acids in our diet, such as palmitic acid. This study has allowed us to identify the epigenetic mechanisms that promote the aggressiveness of metastatic cells. Thanks to this work we have identified a histone methyltransferase, Set1a, whose inhibition erases this prometastatic epigenetic memory and consequently very significantly reduces the metastatic potential in oral squamous carcinomas and melanoma. In collaboration with Prof. Ali Shilatifard (Northwestern University, Chicago) we have developed a specific Set1a inhibitor that we are studying in the context of its antimetastatic activity. We are in the process of carrying out preclinical trials that will indicate its potential in clinical practice.

Furthermore, we have identified the palmitoylome of metastatic cells and one of the proteins involved in this palmitoylation, the DHHC14 protein. We have just obtained an *ERC Proof of Concept* to develop drugs that inhibit this protein and study its antimetastatic therapeutic potential.

3. References

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