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DIPARTIMENTO DI MEDICINA MOLECOLARE  
E DELLO SVILUPPO

### Al collegio docenti del Dottorato in Medicina Molecolare

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Ciclo XXXVII Tutor Antonella Naldini

**Attività scientifica svolta nel 2° anno di Dottorato, Anno Accademico 2022/2023**

#### **Introduction**

The tumor microenvironment (TME) is characterized by hypoxia, a condition in which malignant melanoma cells increase their migratory and invasion capabilities. The Hedgehog pathway, which is normally expressed during embryonal development, is aberrantly activated in cancer and associated to tumor cell proliferation, migration and invasion. Carbonic anhydrases (CAs) are a family of metalloenzymes, which are involved in pH regulation, cell survival and migration, and particularly CAXII emerged as a potential, still poorly studied, target in melanoma.

Dendritic cells (DCs), the most potent antigen presenting cells, are exposed to hypoxia, since they are present in the TME.

#### **Methods**

Melanoma Cell lines (SK-MEL-28 and A375) were cultured in vitro and exposed to normoxia (20% O<sub>2</sub>) and hypoxia (2% O<sub>2</sub>); cells were treated with chemical compounds, Glabrescione B and C22, targeting Hh pathway components Smoothed (SMO) and GLI1. Western Blot, qRT-PCR, Immunofluorescence, Wound healing assay, Zymography, modified boyden chamber, were performed.

Human monocyte-derived DCs were isolated from buffy coats and were exposed to normoxia and hypoxia; JC-1 assay, Western Blot, immunofluorescence and qRT-PCR were performed.

#### **Results**

We demonstrated that the chemical inhibition of SMO and GLI1 resulted in downregulation of CAXII expression under hypoxia, in melanoma cells lines. In addition, we noticed an increased expression of CAXII under hypoxia, highlighting a relationship between the Hh pathway, hypoxia and CAXII itself. We demonstrated that CAXII and the Hh pathway are relevant in melanoma migration and invasion abilities.

With regard to DC, we studied the impact of hypoxia on DC autophagy, focusing our attention on sequestosome-1/p62.

#### **Seminar attended**

VII Siena Think Tank, a vision of I-O call for actions, October 5-7 2023, Siena, promoted by the NIBIT Foundation in collaboration with the Parker Institute for Cancer Immunotherapy and the World Immunotherapy Council

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## Conferences (Abstracts)

Falsini A., Coppola F., Aldinucci C., Naldini A. and Carraro, F. Hedgehog pathway regulates CAXII activity in melanoma cells under hypoxia. SIF 2023, Pisa. 6/09-8/09 2023

Venturella M., Falsini A., Coppola F., Giuntini G., Carraro F., D., Chiesi A., and Naldini A. Hypoxic microenvironment in human melanoma modulates the composition of small extracellular vesicles. . SIF 2023, Pisa. 6/09-8/09 2023

Coppola F., Monaci S., Falsini A., Aldinucci C., Filippi I., Rossi D., Carraro F. and Naldini A. Selective autophagy receptor sequestosome-1/p62 is involved in dendritic cell adaptive responses to hypoxia. SIICA, Verona, 22/05-25/05/2023

## Publications

- Venturella, M., Falsini A., Coppola, F., Giuntini, G., Carraro, F., Zocco, D., Chiesi, A., & Naldini, A. (2023). CA-IX-Expressing Small Extracellular Vesicles (sEVs) Are Released by Melanoma Cells under Hypoxia and in the Blood of Advanced Melanoma Patients. International journal of molecular sciences, 24(7), 6122.  
<https://doi.org/10.3390/ijms24076122>
- Coppola F., Monaci S., Falsini A., Aldinucci C., Filippi I., Rossi D., Carraro F., and Naldini A. SQSTM1/p62 inhibition impairs pro-survival signaling in hypoxic human dendritic cells. BBA research; manuscript under revision.
- Falsini A., Giuntini G., Mori M., Ghirga F., Quaglio D., Cucinotta A., Coppola F., Filippi I., Naldini A., Botta B. and Carraro F. Chemical inhibition of SMO and GLI1 resulted in melanoma migration and invasion impairment: CAXII, hypoxia and Hedgehog pathway interplay. Manuscript in preparation.