MELANOCORTIN RECEPTOR-4, A NEW THERAPEUTIC TARGET OF MELANOMA CELLS

Paola Orlandi[1], Daniela Gentile[1], Teresa Di desidero[1], Francesca Vaglini[2], Carla Pardini[2], Cristina Viaggi[2], Marta Banchi[3], Federico Cucchiara[4], Alessandra Ottani[5], Daniela Giuliani[5], Michele Lai[2], Stefano Aringhieri[2], Marco Carli[2], Shiva Kolachalam[2], Salvatore Guarini[5], Mauro Pistello[2], Marco Scarselli[2], Guido Bocci[6]

[1]Dipartimento di medicina clinica e sperimentale, Pisa - Italy, [2]Dipartimento di Ricerca Traslazionale e delle nuove tecnologia in medicina, Università di Pisa, Pisa - Italy, [3]Dipartimento di medicina clinica e sperimentale, Università di Pisa, Pisa - Italy, [4]Dipartimento di medica clinica e sperimentale, Pisa - Italy, [5]Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Università di Modena e Reggio Emilia, Modena - Italy, [6]Dipartimento di Medicina Clinica e Sperimentale, Università di Pisa, Pisa - Italy

Introduction: Currently, no description of melanocortin receptor-4(MC4R) expression or activity is available in human cancer cells, with the exception of glioblastoma (GBM). In 2018we firstly reported functional MC4R in GBM cancer cells and its inhibition with the only available non-peptide selective antagonist ML0025376. The aim of this study is to evaluate the presence of MC4Rs in melanoma cells and the selective inhibition of their activity through the MC4R antagonist ML00253764alone and in association with vemurafenib.

Methods: MC4R gene and protein expression were performed on human melanoma cells with real-time PCR, western blotting, immunohistochemistry, and immunofluorescence. Proliferation, cell cycle, and apoptotic assays were performed with ML00253764, whereas the synergism of the simultaneous combination with vemurafenib was evaluated by the combination index (CI) method. ERK1/2and Akt phosphorylation were quantified by western blot and ELISA.

Results: The MC4R was well expressed in human melanoma cell lines WM-266-4and A-2058. The ML00253764compound was extremely active on mutated BRAF melanoma cell lines. In fact, after 72h of treatment with ML00253764, it was possible to observe a significant antiproliferative effect already at the concentration of 10 nM both in the A-2058line and in the WM-266-4line with an experimental IC_{50} of 11.1nM and 33.7nM, respectively. Vemurafenib, drug of choice for the treatment of mutated BRAF melanoma, was also very active on both cell lines although with markedly higher IC_{50}s. Indeed, the antiproliferative effects of vemurafenib was higher on WM-266-4cells (IC_{50} 46.6nM, BRAF V600E), while A-2058showed a lower sensitivity to treatment (IC_{50} 526nM, BRAF V600D). Simultaneous treatment of vemurafenib and ML00253764in a fixed molar ratio 1:10 for 72h was performed on the two melanoma cell lines. In both cell lines, the simultaneous combination shows a marked synergism for the fractions of affected cells (Fa) (CI <1). Moreover, the cells treated with ML00253764at a concentration of 10 nM for 72h showed a significant increase of the apoptotic process, with the presence of "shrunken nuclei", characterized by the typical condensation of chromatin which are peculiar to apoptotic cells. Furthermore, the ML00253764compound significantly inhibited the ERK1/2and Akt phosphorylation in both the cell lines.

Conclusions: MC4R is present in melanoma cells and its selective inhibition determined antiproliferative and proapoptotic effects, through the inhibition of ERK1/2and Akt phosphorylation, and the synergistic enhancement of vemurafenib effects. The study has been funded by the Bando Dimostratore Tecnologico of the University of Pisa.

Keywords: MC4R, melanoma, ML00253764, vemurafenib