

## HOW DOES CELLULAR RESPONSE DRIVE TUMORIGENESIS IN MELANOMA CELLS? AN IN VITRO STUDY

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Tumorigenesis includes a series of biomolecular events, not well clarified. Melanoma cells develop adaptive responses to cope specific conditions of the microenvironment, characterized by stress stimuli and a push to fuel continue proliferation. These signals induce the activation of cellular response to stress. The most recent knowledge of melanoma biology focuses on the role of endoplasmic reticulum (ER) stress, autophagy and translational reprogramming. The aim of our study is to analyze the activation of these pathways in an in vitro melanoma model. Metastatic melanoma cell lines BRAF wild-type (wt) and BRAF-mutated were analyzed. We estimated the phosphorylated eIF2alpha (peIF2alpha), LC3 II/I ratio, TFEB and beta catenins levels. Furthermore, confocal microscopy and structure and dynamic studies were used to highlight the localization of peIF2alpha. Our results show higher levels of peIF2alpha in the BRAF-mutated cells, as compared to BRAFwt. The most striking result is the finding of nuclear localization of peIF2alpha. This is the first report of the nuclear localization of peIF2alpha. Dogmatic molecular biology knowledge usually relates eIF2alpha activity into the cytoplasm. Our analysis of the eIF2alpha protein sequence indicated the presence of a predicted bipartite NLS as well as one nuclear export signal NES. The protein also included an S1 domain, typical of RNA-binding proteins. The role of eIF2alpha/peIF2alpha into the nucleus is not clear. However, a possible explanation is provided by studies suggesting the possible link between this translation factor and RNA polymerase. Furthermore, we found higher levels of LC3II/I ratio and TFEB in BRAF-mutated cells, thus supporting the activation of autophagy through the lysosomal pathway. In conclusion, our results suggest a role of peIF2alpha in ER stress response and driving metastatic spread, therefore supporting the prognostic value of eIF2alpha in melanoma.