# XXIII CONGRESSO NAZIONALE IMI

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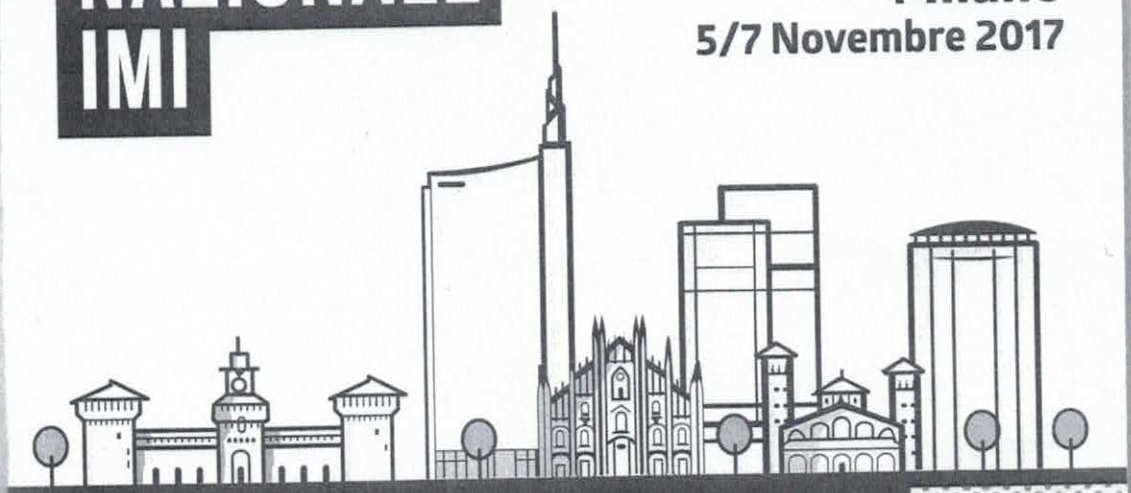

## PROGRAMMA

intergruppo melanoma italiano
imi

**Intergruppo Melanoma Italiano**

GIUSEPPE PALMIERI PRESIDENTE IMI
MICHELE DEL VECCHIO PRESIDENTE CONGRESSO

Area tematica di riferimento: genetica e cancerogenesi

**Stress response pathways driving tumorigenesis in melanoma cell lines**

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**Background**

Malignant transformation and tumorigenesis include a complex series of cellular and biomolecular events which are not yet completely known. Tumor cells develop adaptive responses in order to cope particular conditions of the tumor microenvironment, characterized by stress conditions and by a dysregulated proliferation. These signals induce the activation of cellular response to stress. The aim of our study is to evaluate the activation of these stress pathways in an *in vitro* cellular model.

**Methods**

Melanoma cells, BRAF wt and BRAF-mutated, extracted from primary or metastatic tumors, were used as cellular model [1,2]. We estimated the phosphorylated status of eIF2-α (peIF2-α), the LC3 II/I ratio and the TFEB basal levels by Western blotting. Furthermore, we used confocal microscopy and mass spectrometry in order to highlight the localization of peIF2-α .

**Results**

Our results show higher levels of peIF2-α in the metastatic BRAF-mutated melanoma cells as compared to the BRAF wt primary BRAF-mutated melanoma cells [1]. The most striking result of our work is the finding of nuclear localization of peIF2-α, including the cell lines from the primary lesion. Dogmatic molecular biology knowledge usually relates eIF2-α activity into the cytoplasm. Furthermore, we found in all BRAF-mutated cells with respect to BRAF wt metastatic melanoma cells, higher LC3II/I ratios and TFEB levels, markers of increased autophagy [1].

**Conclusions**

This is the first report of the nuclear localization of peIF2α, known for its crucial role in ER stress response and in driving metastatic spread of melanoma. Further study will be performed in order to evaluate its activity into the nucleus and to improve the emerging knowledge relating to eIF2-α activity in the nucleus.

In addition, we found higher levels of autophagy in BRAF-mutated cell lines, leading to support the activation of autophagy through the lysosomal pathway in our cell lines.

## References:

[1] New insight into the role of metabolic reprogramming in melanoma cells harboring BRAF mutations.Ferretta A,et al..Biochim Biophys Acta. 2016 Nov;1863(11):2710-2718. doi: 10.1016/j.bbamcr.2016.08.007. Epub 2016 Aug 16.

[2] Three novel human sporadic melanoma cell lines: signaling pathways controlled by MC1R, BRAF and β catenins. Zanna P et al..J Biol Regul Homeost Agents. 2013 Jan Mar;27(1):131-41

# Stress response pathways driving tumorigenesis in melanoma cell lines

Anna Ferretta[a], Stefania Guida[b], Michele Manganelli[a], Immacolata Maida[a], Tiziana Cocco[a], Anna Gallone[a], Stefania Tommasi[c], Amalia Azzariti[c], Michele Guida[c], Gabriella Guida*[a]

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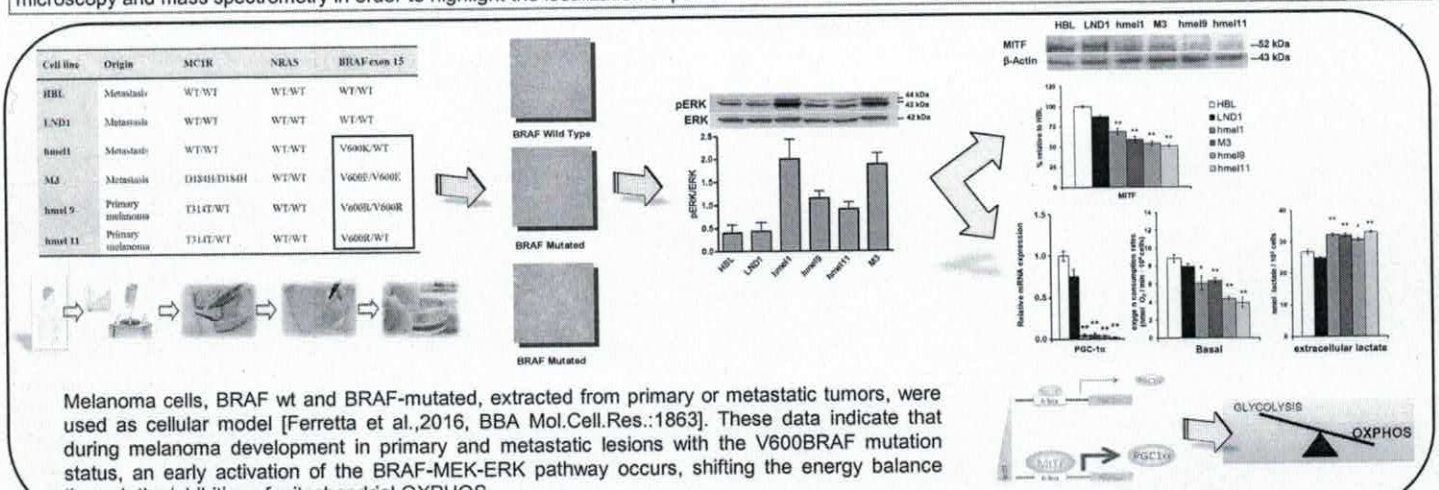
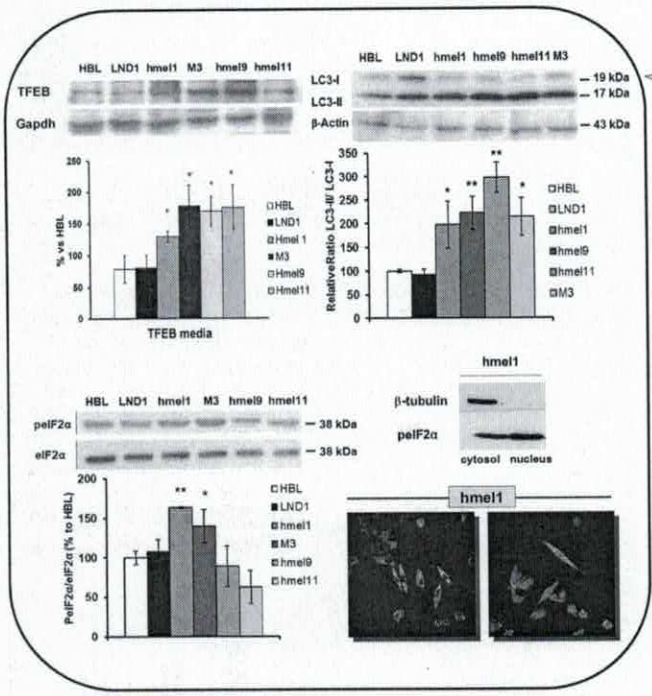
**AREA TEMATICA DI RIFERIMENTO: genetica e cancerogenesi**

**Background** Malignant transformation and tumorigenesis include a complex series of cellular and biomolecular events which are not yet completely known. Tumor cells develop adaptive responses in order to cope particular conditions of the tumor microenvironment, characterized by stress conditions and by a dysregulated proliferation. These signals induce the activation of cellular response to stress. The aim of our study is to evaluate the activation of these stress pathways in an *in vitro* cellular model.

By Western Blotting analyses we estimated the phosphorylated status of eIF2-α (peIF2-α), a translation initiation factor that functions in the early steps of protein synthesis, the LC3 II/I ratio and the TFEB basal level, autophagy and lysosomal stress biomarkers, respectively. Furthermore, we used confocal microscopy and mass spectrometry in order to highlight the localization of peIF2α.




Melanoma cells, BRAF wt and BRAF-mutated, extracted from primary or metastatic tumors, were used as cellular model [Ferretta et al.,2016, BBA Mol.Cell.Res.:1863]. These data indicate that during melanoma development in primary and metastatic lesions with the V600BRAF mutation status, an early activation of the BRAF-MEK-ERK pathway occurs, shifting the energy balance through the inhibition of mitochondrial OXPHOS.





## RESULTS

Our results show higher levels of phosphorilated eIF2-α (peIF2α) in the metastatic BRAF-mutated melanoma cells as compared to the BRAFwt primary BRAF-mutated melanoma cells. The most striking result of our work is the finding of nuclear localization of peIF2α, including the cell lines from the primary lesion. Dogmatic molecular biology knowledge usually relates eIF2-α activity into the cytoplasm.

Furthermore, we found in all BRAF-mutated cells with respect to BRAF wt metastatic melanoma cells, higher LC3II/I ratios and TFEB levels, markers of increased autophagy.

## CONCLUSION

**ER stress** and **autophagy** play a key role in homeostasis as well as pathological processes. It is known that phosphorylation of the α- subunit of the translation initiation factor eIF2 at serine 51 **(peIF2α) is a master regulator of cell adaptation to various forms of stress**, acting as a molecular switch that dictates either cell survival or death .

In our study we highlight an increase of peIF2α in MM but not in primary $^{V600}$BRAF cells, thus delineating autophagy activation by ER stress as a pro-survival mechanism in peIF2α-proficient cells (MM $^{V600}$BRAF cells), suggesting peIF2α as a marker of a more aggressive phenotype in melanoma.