Characterizing the clinical relevance and the mechanism underlying TRIB2-mediated drug resistance to MEK inhibitiors in the context of melanoma

Rendicontazione

Informazioni relative al progetto

TRIBBLES

ID dell'accordo di sovvenzione: 748585

Sito web del progetto

DOI
10.3030/748585

Progetto chiuso

Data della firma CE
6 Marzo 2017

Data di avvio
1 Settembre 2018

Data di completamento
31 Agosto 2020

Finanziato da
EXCELLENT SCIENCE - Marie Skłodowska-Curie Actions

Costo totale
€ 160 635,60

Contributo UE
€ 160 635,60

Coordinato da
UNIVERSIDADE DO ALGARVE
Portugal

Periodic Reporting for period 1 - TRIBBLES (Characterizing the clinical relevance and the mechanism underlying TRIB2-mediated drug resistance to MEK inhibitiors in the context of melanoma)

Periodo di rendicontazione: 2018-09-01 al 2020-08-31

Sintesi del contesto e degli obiettivi generali del progetto

Malignant melanoma is a highly aggressive cancer that arises from the transformation of melanocytes, the pigment-producing cells within the basal epidermal layer in human skin. Malignant melanoma accounts for only 5% of all skin cancers, yet is responsible for 80% of skin cancer deaths.While current therapeutic approaches (MEK inhibitors, BRAF inhibitors and immune checkpoint inhibitors) have yielded unprecedented clinical responses, the majority of patients fail to respond or acquire resistance to these treatments. Therefore, investigating the cause of drug resistance is key to the development of better therapies.
Importantly, the Link lab discovered a novel mechanism of resistance to a broad spectrum of drugs in clinical trials/use for melanoma therapy mediated by the kinase-like protein Tribbles homolog 2 (TRIB2).TRIB2 belongs to a wider family of proteins named the TRIBBLES protein family which are highly conserved during evolution. TRIB2 mediates AKT activation resulting in increased MDM2 activity and FOXO inhibition. As a consequence, the expression of FOXO target genes, which could lead to drug induced apoptosis, is attenuated by TRIB2. AKT is an important kinase that belongs to the PI3K pathway, frequently mutated and hperactivated in several human cancers.
This proposal sets out a strategic multidisciplinary approach to (1) characterize the mechanisms involved in TRIB2 mediated resistance to MEK inhibitors and (2) evaluate the clinical relevance of our findings. The expected
results will be clinically relevant as they will allow stratifying patients into responders and non-responders to anticancer agents in a personalized medicine setting, leading to the development of co-treatment strategies.

We have used the gene editing technology, CRISPR-Cas9, to generate TRIB2 Knock-out cells and analyse changes in gene expression dependent on TRIB2 status. Following, we identified compounds capable of reversing TRIB2-mediated resistance. With this approach, these compounds club be potentially be used in the clinic to overcome therapeutic resistance. We found that the naturally occurring alkaloids - Harmine and Piperlongunime -could inverse the gene expression profile induced by TRIB2 expression. Moreover, both compounds are able to promote FOXO nuclear translocation and induce the transcription of FOXO target genes.

Lavoro eseguito dall'inizio del progetto fino alla fine del periodo coperto dalla relazione e principali risultati finora ottenuti

Work Package 1 (WP1)
WP1 consisted of research activities directed towards O1 and O2: Generation of TRIB2 knockout (KO) cell line using CRISPR-Cas9 technology. The ER has:
• Completed a wide melanoma cell line screening for TRIBBL protein and selected UACC-62 as the cell line to be manipulated by CRISPR-Cas9 technology.
• Successfully cloned TRIB2 guided RNA (gRNA) and generated UACC-62 TRIB2 KO. Additionally,

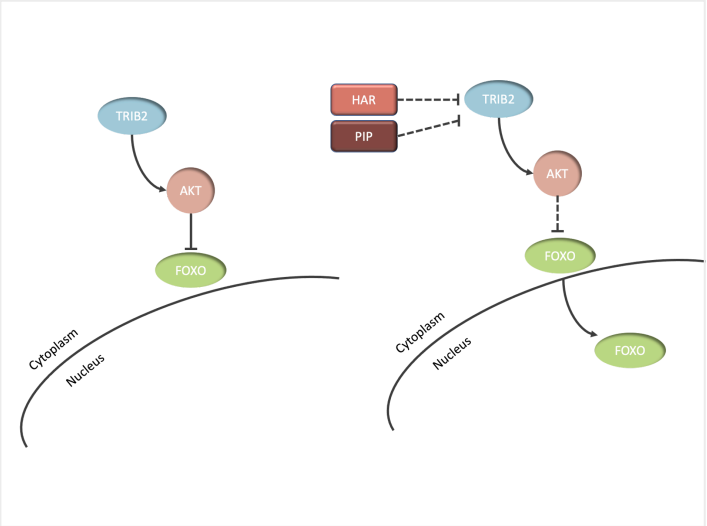
the ER has cloned two independent gRNA for TRIB3 and TRIB1 following the same procedure as with TRIB2 cloning.
• UACC-62 TRIB2-KO and parental cells have been treated with Trametinib, Afatinib, BAY-9766 and BEZ-235.
• Cell lines were analyzed for percentage of cell death, cell cycle and apoptosis.
• TRIBBL's family members protein levels upon drug treatment.

Work package 2 (WP2)
Consisted of research activities directed towards objective 2 (O2):
• RNA-Seq data was analyzed in collaboration with Isabel Duarte, PhD (CBMR, Faro). With this analysis the ER was able to pinpoint the genes that were differentially expressed between cell lines overexpressing TRIB2 and parental match.
• The ER has matched the data with a publicly available database – Connectivity Map (cMAP) and generated a candidate list of natural compounds that could inverse TRIB2 expression profile.
• Validate RNA-Seq results by quantitative real-time PCR (qRT-PCR)
• Validate by western-blot candidate genes
• Validate effect of Harmine (HAR) and Piperlongumine (PIP) - selected natural compounds from cMAP- on cell lines generated in WP1
• Analyse FOXO (downstream of TRIB2) subcellular localization upon HAR and PIP treatment.
• Analyse TRIB2, TRIB3 and AKT protein complex association by proximity luciferase assay.

Work package 3 (WP3)
Consisted of research activities directed towards objective 3 (O3):
• The ER conducted analysis of TRIB2, TRIB3 and TRIB1 mRNA levels on publicly available database of melanoma patients
• Generated survival data depending on TRIBBLE's mRNA levels.
• Generate TRIBBLE's specific antibodies.
• Validate antibodies for Western blot and immunohistochemistry.

Progressi oltre lo stato dell'arte e potenziale impatto previsto (incluso l'impatto socioeconomico e le implicazioni sociali più ampie del progetto fino ad ora)

The potential impact of the project results is significant, since have permitted to identify two natural compounds that counteract TRIB2 induced resistance. As a result, the ER has identified a potential mechanism by which cancer patients with high level of TRIB2 expression are not responsive to therapy when compared to patients with low TRIB2 expression. This mechanism involves regulation of FOXO proteins subcellular localization. Trapping FOXO protein in the nucleus of the cell has been in very recent years a revisited cancer treatment strategy by different companies. The results of this project support a combined treatment strategy that depend on TRIB2 status and modulate FOXO localization.



h2020.png

Ultimo aggiornamento: 19 Luglio 2021

Permalink: https://cordis.europa.eu/project/id/748585/reporting/it