EVALUATING THE EFFECTIVENESS OF PRECISION THERAPIES AIMED AT RECURRENT GENETIC ALTERATIONS IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

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1. Project summary

Malignant peripheral nerve sheath tumor (MPNST) is a very aggressive type of soft tissue sarcoma. Approximately half of MPNSTs develop in sporadic patients and the other half in patients with neurofibromatosis type 1 (NF1), an inherited disease with a predisposition to cancer. MPNST affects both young people and adults and has a very poor prognosis due to its aggressiveness and metastatic potential. Full resection with wide margins is the most effective therapy for MPNSTs, but it is not always feasible. Radiotherapy and chemotherapy are of little benefit in many cases and there is a clear need to find new treatments.

The genome of MPNSTs is highly altered, but generally they have three tumor suppressor genes (*NF1*, *CDKN2A*, *SUZ12*) completely inactivated, since the loss of function of these genes seems essential for their development. This is very positive because there are drugs whose biochemical pathways are altered by the loss of these genes as therapeutic targets. The objective of this project was to understand the importance of these three genes in the biology of MPNSTs and to evaluate the efficacy of drugs against their absence of function in a precision medicine strategy, both in human tumors implanted in mice, and in a clinical context in patients. It has been a project that has brought together basic, preclinical and clinical research, from a multidisciplinary team made up of biologists, bioinformaticians, geneticists, oncologists and pathologists, from different hospitals and research centers.

Thanks to this project, we have achieved a better genomic characterization of MPNSTs, key in the search for precision medicine treatments. In addition, we have been able to understand the role of *the NF1*, *CDKN2A* and *SUZ12 genes* in the progression towards malignancy, identifying the loss of function of the *SUZ12* gene as a key to the glial-mesenchymal transition. This has opened the door to the identification of biomarkers of progression, which could be used for the monitoring and follow-up of people with neurofibromatosis type 1. On the other hand, we have identified that a precision medicine treatment based on the combination of MEK and BET inhibitors is highly effective in human MPNST tumors implanted in mice. These results have made it possible to start using this therapy in the first pilot studies in humans, in children who have developed one of these tumors.

2. Results

At the level of basic research

We have made a very exhaustive characterization of the genome of MPNSTs from complete genome and transcriptome sequencing studies. We have been able to obtain a precise genomic definition of MPNSTs with a classical histology, which will complement the histological characterization when making a differential diagnosis of MPNSTs. In addition, we have generated a genomic repository, which will be cross-referenced with clinical and therapeutic response data in mouse models and humans. Using a cellular model based on induced pluripotency stem cells (iPSC) and the use of genome editing tools (CRISPR), we have generated different cell lines that carry inactivation of different tumor suppressor genes (*NF1*, *CDKN2A*, *SUZ12* (PRC2)). We have discovered that there is a constriction in cell viability, which imposes an order in the loss of these three genes. In addition, we have discovered that the inactivation of the two proteins encoded by the *CDKN2A* gene (p14 and p16) is necessary for a neurofibroma to progress to an MPNST. Finally, we have been able to demonstrate that the loss of function of the PRC2 complex induces a change in cell identity, from a glial to a mesenchymal identity, exactly what is observed in the transition from a plexiform neurofibroma to an MPNST.

At the level of preclinical research

This project has in-depth investigation of the possibilities of using drugs that target the effects of the loss of function of the three tumor suppressor genes most recurrently inactivated in MPNSTs. From an extensive library of drugs, the combination possibilities of 29 compounds, consisting of MEK inhibitors, CDK4/6 inhibitors and BET inhibitors (bromodomain) have been saturated. The combination of in vitro and in vivo studies has identified the combination MEKi + BETi as the best drug combination for MPNSTs, both sporadic and associated with neurofibromatosis type 1 (NF1). In fact, the results of response to treatment with MEKi+BETi in vivo in human PDOX models are the best reported so far in the literature, and robustly reaffirm the results in murine tumors, obtained from genetically modified models. In fact, two pilot co-clinical projects of personalized medicine have been carried out, where it has been possible to generate a human PDOX and treat it with these (and other) drug combinations, while giving the first line of treatment to patients. These two personalized in vivo studies, MEKi + BETi,

are the best identified so far and stand as a therapeutic possibility of compassionate use for MPNSTs in case the first line of treatment does not work.

At the level of clinical research

The circuits for collecting liquid biopsy have been established, the first samples collected and preserved from different patients and the nanopore sequencing analysis strategy has been designed. Multiple multidisciplinary committees of MPNST tumors (tumor molecular boards) have been held where the co-clinical trials carried out and the clinical evolution of the patients have been monitored, together with the genomic characterization and the results of treatments in PDOX of mice. Based on the in vivo preclinical results, the pharmaceutical industry has been contacted to have different drugs, in particular, MEK and BET inhibitors. Currently, co-treatments are being carried out with MEK and BET inhibitors as compassionate use in the first patients.

3. Relevance with possible future implications

Use of the combination of MEK and BET inhibitors as compassionate use in patients with MPNST.

The results obtained with the precision medicine platform have been reinforced with those obtained with personalized treatments in the co-clinical trials that have been carried out. In both cases treated in the last year, the MEKi-BETi combination has continued to give the best results, reducing the tumor by up to 60% during the three weeks of treatment, and using a treatment regimen that has been well tolerated at the level of toxicity. These results have made it possible to inform the respective tumor committees (molecular tumor boards) about the potential use of this combination as a compassionate use for the respective patients. The MEKi used, selumetinib, has already been approved for use by the American drug agency (FDA) and the European drug agency (EMA). The BETi obtained in a pharmaceutical company has already undergone toxicity and dosage studies in humans. Thus, a patient is already receiving this combination of drugs as compassionate use, and their tolerability and effect are being monitored. The first results, still preliminary, are positive, both for the tolerance of the treatment at the level of toxicity, and for the brake on tumor progression. Clearly, this combination is promising and its effectiveness in new cases will surely have to be explored.

New drug screenings

The development of new iPSC lines containing mutations in the three tumor suppressor genes responsible for initiating the transformation of a plexiform neurofibroma into an MPNST represents a reliable model of this progression. These isogenic lines are already being used in large-scale drug screening for MPNSTs with a collaboration we have initiated with the NIH (USA). These drugs will be candidates to be tested in our in vivo models of MPNSTs (preclinical subproject) and potentially applicable to MPNST developed in patients.

In vivo platform expansion and gain of predictive capacity in new tumors.

Another application of this project is that it has made it possible to expand the precision medicine platform. This will allow us to have a broader predictive response capacity, with a greater palette of tumors with different genomic alterations.

Biomarkers for the diagnosis and monitoring of patients

The precise genomic definition of MPNSTs obtained will complement the histological characterization when making a differential diagnosis of MPNSTs. At the level of studies with different lines derived from induced pluripotency (iPSC) cells edited by CRISPR, we have been able to identify progression biomarkers, which will have to be validated in patients.

Repository for personalized and precision medicine

We have generated a repository that contains clinical, genomic and therapeutic response data in mouse models and in humans. This repository should allow us to have predictive capacity before the appearance of new MPNST in children or adults, which would allow us to identify the potential best treatments in each case.

4. Scientific bibliography generated

Published articles

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