DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

Proposta di assegnazione di una borsa di Dottorato

Titolo:

English:

Pro-apoptotic and anti-angiogenic effects mediated by new dualsteric agonist for M2 muscarinic receptors in glioblastoma of cancer stem cells.

Italian:

Effetti pro-apoptotici e anti-angiogenici mediati da un nuovo agonista dualsterico del recettore muscarinico M2 in cellule staminali tumorali di glioblastoma

Proposed Supervisor:

Prof.ssa Ada Maria Tata

DESCRIZIONE DELLA RICERCA (max 2 pagine)

Objectives:

Glioblastomas (GBM) are the most aggressive form of primary brain tumors in humans. Their poor prognosis largely derives from their high growth potential and migratory ability that prevents resolutive surgical resection. The low susceptibility to apoptosis displayed by glioblastoma cells makes radiotherapy and chemotherapy ineffective. High growth rate and invasiveness thus appear as first targets to counteract in these tumors. A key feature of malignant gliomas is their cellular heterogeneity. In particular, the presence of an undifferentiated cell population defined Glioblastoma Stem cells (GSCs) has been reported. Increased expression of anti-apoptotic and chemo-resistance genes in GCSs subpopulation, favors their high resistance to a broad spectrum of drugs. GSCs show high resistance also to radiotherapy, because of their ability to repair DNA damage by radiation. GSCs are considered responsible for tumor growth and invasiveness, they are able to evade the most common cancer therapies and are considered the main cell population responsible for the beginning of the development of neoplastic process and of recurrence formation. For these reasons, the identification of new drugs able to inhibit the proliferation and survival of GSCs are currently considered to have an important clinical relevance. Previously studies have shown that cell lines of glioblastoma multiforme, U87 and U251, express muscarinic receptors for acetylcholine. In particular the activation of muscarinic M2 receptor by orthosteric agonist Arecaidine (APE) caused a significant decrease of cell proliferation and survival. Similarly in GSC obtained from biopsies of patients we have confirmed the cytostatic effect of APE (Ferretti et al., 2012, 2013; Di Bari et al, 2018). Moreover APE induce also cytotoxic effects in particular in GB cell lines p53 mutated or deleted (Di Bari et al, 2015). Recently we have tested a new dualsteric agonist for M2 receptor (N8-Iper) that show high selectivity and more efficiency than APE and that is able to activate M2 receptor at very low concentration (Cristofaro et al, 2018). Aim of this project will be to evaluate the ability of N8-Iper to negatively modulate cell survival in GSCs; its possibile cytotoxic effects in terms of oxidative and ER stress induction and its ability to negatively modulate neo-angiogenesis promoted by GSCs will be also investigated.

Scopo del presente progetto e (risultati preliminari) .

M2 muscarinic receptor activation has a negative effects on cell proliferation and survival in different tumor types (i.e. urothelial cancer cells, neuroblastoma, breast cancer) including glioblastoma. Our previous studies have demonstrated the ability of the orthosteric agonist of M2 receptor to affect cell cycle progression and survival in glioblastoma cell lines and in GSCs,

inducing cytotoxic and genotoxic effects. More recently we have tested a new dualsteric agonist for M2 receptors able to counteract both cell proliferation and survival of glioblastoma cells at lower doses than orthosteric agonist. In order to clarify the mechanism downstream the M2 receptors activation after N8-iper, we propose to study the possible ability of the dualsteric agonist to induce oxidative stress and/or ER stress in different GSC cell lines. Glioblastoma produces a microenvironment favourable for tumour cell survival inhibiting the immune system activity and promoting the neo-angiogenesis. Muscarinic receptors are able to promote the pro-inflammatory cytokine production and can control the endothelial cell proliferation and differentiation. Starting from this evidence, we'll be evaluate the ability of the N-8 iper to modulate cytokines production and neo-angiogenesis. In this context we'll also evaluate the secretoma produced by GSCs in absence or in presence of M2 dualsteric agonist stimulation.

Stato delle conoscenze

Muscarinic receptors appear to be involved in brain tumors. Patients with brain tumors often show altered levels of ACh in their cerebrospinal fluid; moreover the presence of muscarinic receptors in astrocytoma and in glioma cell lines has been widely described. In astrocytoma cell lines ACh has been shown to stimulate proliferation and activate both ERK1/2 and PI3K pathways, mediated through M3 muscarinic receptor activation. Moreover, as assessed in *in vitro* studies, it has been demonstrated that the M2 muscarinic agonist APE is capable to arrest in glioblastoma cell lines (U87MG and U251MG) and GSCs cell cycle progression with a significant decrease of cells in S phase. Moreover, M2 receptor activation reduces cell survival, inducing a dramatic apoptosis in both cell lines. The agonist effect of APE on M2 receptors has been confirmed both by pharmacological competition studies and by M2 receptor silencing by siRNA; in both experimental conditions the arecaidine effect was completely reverted. APE was also able to induce cytotoxic and genotixic effects by ROS production (Di Bari et al, 2015).
More recently, in spite of a high homology between muscarinic receptors, the identification of allosteric modulators of cholinergic receptors underpins a real possibility of obtaining new pharmacological drugs able to selectively modulate cholinergic receptor activity by binding to allosteric sites. In this respect, rationally designed ligands showing a simultaneous orthosteric/allosteric binding mode at mAChRs represent novel, powerful pharmacological tools to better investigate receptor activation or inhibition (Matera and Tata, 2014). Recently we have characterized the effect of N8-iper, a new dualsteric agonist for M2 receptors that show high selectivity and more efficiency than APE and that is able to activate M2 receptor at very low concentration (Cristofaro et al, 2018). These results suggest that N-8 iper may be and interesting therapeutic drug for glioblastoma therapy, moreover reducing the possible side effects depending on the high doses of APE. In this context a better characterization of the effects mediated by N8-iper results of great relevance.

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Fondi attualmente disponibili per svolgere il programma di ricerca.

Progetti medi Ateneo **2017, 2018**
Progetto MIUR Network CIB 2018*: NGS e nuove tecnologie per il riposizionamento di farmaci e la terapia di precisione.*

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