

POSTERS

DYNAMICS AND INTRACELLULAR TRAFFICKING OF M2R-DUALSTERIC AGONIST COMPLEX INTO GLIOBLASTOMA CELLS

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The M2 muscarinic receptor is a typical G protein-coupled receptors with inhibitory properties. Behind its canonically physiological role in the nervous system, previous studies demonstrated that its selective activation causes inhibitory effects in several tumors including Glioblastoma (GBM) [1]. Recently, we investigated the role of Naphthalimide derivatives in inhibiting GBM cell proliferation, promoting oxidative stress, mitochondria alteration and cell death by autophagy process [2]. These effects were produced when M2 muscarinic receptor subtype (M2R) binds dualsteric agonist N8, a specific bitopic agonist able to bind both allosteric and orthosteric binding sites of M2R. Its binding to the receptor induces "biased agonism" downstream receptor, promoting strong effect upon low-dose treatment. Remarkably, we recently used an auto-fluorescent version of N8 (fluo-N8) very useful to better understand the interaction with M2R and the intracellular trafficking of the drug-receptor complex, analysing the drug-receptor complex to expand our knowledge about M2R activation mechanisms. Bioinformatic and binding analysis allowed the identification of the binding pockets and confirmed the pharmacological properties of the two dualsteric agonists. Immunofluorescence analysis al-

lowed to monitoring receptor dynamics and intracellular trafficking at different stimulation time-points, observing the strong expression of M2R into plasma membrane after agonist stimulation and the translocation into the cytoplasm by β -Arrestin1 interaction. Moreover, thanks to the autofluorescence features of fluo-N8, it was also possible to investigate the drug-receptor complex, demonstrating the ability of fluo-N8 to be internalised in the cells through the M2R, and its co-localization at different time-points, with different cellular organelles and compartments, including vesicle-like structures, cis-Golgi and mitochondria. Interestingly no significant effects were evident in normal human astrocyte when treated with these M2 ligands.

References

1. Cristofaro I, Spinello Z, Tata AM. Cholinergic system and its role in oligodendrocyte development and function. *Neurochem Int.* 2018 Aug;118:52-70.
2. Guerriero C, Manfredelli M, Matera C, Iuzzolino A, Conti L, Dallanoce C, et al. M2 Muscarinic Receptor Stimulation Induces Autophagy in Human Glioblastoma Cancer Stem Cells via mTOR Complex-1 Inhibition. *Cancers (Basel).* 2024;16(1):25.