Anđela Petrović

Uticaj inhibicije Rag GTPaza gliciretinskom kiselinom na mTORC1 u MG ćelijskoj liniji humanog osteosarkoma i CRL2066 ćelijskoj liniji tumora pluća

Proteinski kompleks mTORC1 ima više uloga u ćeliji, kao što su regulisanje sinteze proteina, procesa formiranja ribozoma, anabolizma proteina i unosa nutrijenata kao i kontrola autofagije i odgovora na unutrašnji stres. Jedan od signalnih puteva preko kog se navedene uloge ostvaruju jeste signalni put aminokiselina i nutrijenata u lizozomu. mTORC1 se vezuje za regulatorski kompleks na lizozomu uz pomoć Rag GTPaza čiji je zadatak da menjaju GDP/ GTP stanje regulatorskog komlepksa kada su u lizozomu prisutne aminokiseline. Ovakvim vezivanjem dolazi do aktivacije mTORC1, a daljim fosforilacijama do početka translacije. Ranijim istraživanjima na *Drosophilia melanogaster*, utvrđeno je da usled smanjenja količine aminokiselina dolazi i do smanjenja veličine ćelije, slično kao i pri mutiranju TOR gena. Takođe, u istraživanju u kom su ćelije tretirane urzolinskom kiselinom primećeno je da je izostala relokalizacija mTORC1 kompleksa iz citoplazme na membranu lizozoma, što je ključno za njegovu aktivaciju i čime se aktivnost mTORC1 povezuje sa aktivnošću Rag GTPaza.

U ovom radu je ispitivan uticaj inhibicije Rag GTPaza gliciretinskom kiselinom ekstrahovanom iz suvog korena sladića *Glycyrrhiza glabra* i uticaj te inhibicije na aktivnost mTORC1 kompleksa u ćeliji. Ispitivan je efekat različitih koncentracija gliciretinske kiseline na ćelije sa ili bez dodatka aminokiseline leucin, kao i sa ili bez dodatka insulina. MTT analizom merenja vijabiliteta ćelija pokazano je da se vijabilitet ćelija osteosarkoma i tumora pluća proporcionalno smanjuje pri tretmanu sa 30 μM, 40 μM i 50 μM gliciretinskom kiselinom. Ovi rezultati potencijalno mogu biti značajni pri borbi protiv tumorskih ćelija, kao i za kontrolisanje autofagije i translacije. Takođe, povezivanjem ovog istraživanja sa prethodnim, otkrivanjem potencijalnog mehanizma dejstva i polovine maksimalne inhibitorne koncentracije (eng. the half maximal inhibitory concentration, IC50IC50) moguće je dalje proučavati potencijalno antitumorsko dejstvo.

The influence of Rag GTPases Inhibition by Glycyrrhetinic Acid on mTORC1 in MG Human Osteosarcoma and 2066 Lung Cancer Cells

The protein complex mTORC1 has numerous roles in the cell, such as the regulation of protein synthesis, the process of ribosomes forming, protein anabolism and nutrient intake, as well as the control of autophagy and response to internal stress. One of the signaling pathways through which these roles are derived is the signal pathway of amino acids and nutrients in lysosomes. The protein complex mTORC1 binds to the regulatory complex on lysosomes with the help of a Rag GTPases, the task of which is to change the GDP/GTP state of the regulatory complex when there are amino acids presented in the lysosomes. This binding leads to the activation of mTORC1 and further phosphorylations to the beginning of translation. Earlier researches on *Drosophila melanogaster* found that due to the reduction in the amount of amino acids, the cell size decreased, similar to the mutation of the TOR gene. Also, in previous studies it has been shown that in cells that were treated with ursolic acid there was no relocalization of the mTORC1 complex

Anđela Petrović (2000), Beograd, Vidikovački venac 79/58, učenica 3. razreda XIII beogradske gimnazije

MENTOR: Jovan Traparić, student Biološkog fakulteta Univerziteta u Beogradu

from the cytoplasm to the lysosomal membrane, which is crucial for its activation and by which the activity of mTORC1 is associated with the activity of Rag GTPases.

In this study, the influence of Rag GTPases inhibition by glycyrrhetinic acid extracted from the dry root of *Glycyrrhiza glabra* and the effect of this inhibition on the activity of the mTORC1 complex in the cell was examined. Also, the effect of different concentrations of glycyrrhetinic acid on cells with or without leucine, as well as with or without insulin addition, was examined. MTT analysis of cell viability measurements showed that the viability of osteosarcoma and lung tumor cells decreased proportionally when cells were treated with 30 μM, 40 μM and 50 μM glycyrrhetinic acid. These results can potentially be significant in the fight against tumor cells, as well as for controlling cell autophagy and translation. Also, by linking this study to previous ones, by knowing the potential mechanism of action and half of the maximum inhibitory concentration (IC50), it is possible to further study the potential antitumor effect.