Abstract

Glucocorticoids (GCs) are steroid hormones, which exert their actions through glucocorticoid receptor (GR). GCs regulate many cellular functions such as metabolism, apoptosis, immune responses, and cell growth. Glucocorticoid receptor acts as transcriptional factor, transactivating or transrepressing the expression of a variety of target genes. Glucocorticoids are widely used in medicine, due to their anti-inflammatory and tissue spesific apoptotic activities. However, chronic use of glucocorticoids can also lead to negative side effects, such as diabetes, loss of muscle mass, and osteoporosis. For this reason, there is high need for selective GR agonists (SEGRAs), which will dissociate transrepression from transactivation activities. GR is found to exert nuclear, cytosolic, cell membranous, but also mitochondrial localization. Mitochondrial GR can regulate mitochondrial transcription by direct binding to specific hormone responsive elements within the D-Loop regulatory region of the mitochondrial DNA. In addition via interaction with other regulatory molecules can affect mitochondrial dependent apoptosis. In this study, 52 Triterpenoids were examined for their ability to activate GR nuclear translocation and act as SEGRAs. Tetracyclic triterpene protopanaxadiol (**PPD**) and protopanaxatriol (**PPT**), derivatives of boswellic acid [11-keto beta Boswelic acid (**KBA**), 3-0-Acetyl-11-ketobeta Boswelic acid (**AKBA**), Boswelic Acid alpha (**α-BA**), Boswelic Acid beta (**β-BA**), Acetyl alpha-boswelic acid (**α-ABA**), Acetyl beta-boswelic acid (**β-ABA**)], the α-amyrin and its derivatives and derivatives of betulinic acid were further analysed for their dissosiative GR activity. Thus, immunohistochemistry, luciferase reporter gene assays, immunoblotting analysis (Western Blot) and/or induced-fit docking analysis were applied for that purpose. PPD, PPT and boswellic acid derivatives, showed comparable to DEX efficiency to bind to the ligand binding domain of GR. Compounds KBA, AKBA, α-BA, PPT and to a lesser extent compounds β-BA, α-ABA and β-ABA, PPD were capable of inducing GR nuclear translocation, suppression of NFκ-B transcriptional activity in HeLa and HEK293 cells lines (positive GR cells), but not in COS.7 cells (negative GR cells), indicating involvement of GR in this processes. Moreover, overexpression of GR, in luciferase assays, revealed additive effect on the DEX-induced NF-κB transcriptional repression by the KBA and AKBA compounds. Also, in the presence of the above mentioned triterpenoids, no transactivational activity of GR was observed, whereas repression of the dexamethasone-induced (DEX) transcriptional activity of GR was observed. Consequently, PPD, PPT and boswelic acid derivatives could be considered as SEGRAs.

In addition, PPD, PPT and boswelic acid derivatives were capable in inducing apoptosis in HeLa and HepG2 cells. It is also noteworthy that upon coadministration of AKBA and α-BA compounds with DEX, enhanced apoptotic activity was observed indicating additive effects of AKBA and α-BA with DEX. Moreover, α-amyrin and its derivatives and betulinic acid derivatives were not capable of inducing GR nuclear translocation and suppression of NF-κB transcriptional activation. No effect on GR transcriptional activation was observed by the compounds. Moreover, betulinic acid derivatives and α-amyrin exhibied transrepression of the dexamethasone-induced (DEX) transcriptional activity of GR, in HeLa cells, indicating potent hypoglycemic ativities of

the compounds. Furthermore, due to the important and not well-defined role of mitochondrial GR, we focused on the characterization of the GR interacting proteins in mitochondria. Proteins examined were key molecules in the regulation of the mitochondrial metabolism and transcription. Applying co-immunoprecipitation studies, by the use of spesific antibodies, we showed that proteins such as 1) 70 Kilodalton Heat Shock Protein (HSP70), 2) 90 Kilodalton Heat Shock Protein (HSP90), 3) Peroxisome proliferator-activated receptor alpha (PPARα), 4)Pyruvate dehydrogenase (PDH), 5) Voltage dependent anion channel (VDAC), 6) Mitochondrial transcription factor (MtfA), and the anti-apoptotic protein BCl2, are GR interactuing proteins. Among them, the proteins PDH, HSP70 and PPARα exhibited strong interactions with GR. PDH is a key enzyme, responsible for the observed metabolic reprogramming in several types of cancer cells. Thus, the observed GR-PDH interaction prompted us to examine, the role of mtGR in cancer. For this reason, NOD-SCID mice (lacking B- and T-cells), and NSG mice, [lacking B-, T-, and NK (Natural Killer cells)-cells] were inoculated with hepatocarcinoma HepG2 stable cell lines overexpressing a mitochondrial targeted green fluorescence protein (HepG2mtGFP) or a mitochondrial targeted GFPGR (HepG2mtGFPGR). Tumors produced in mice, vaccinated with HepG2mtGFPGR cells, were appeared earlier than tumors in control HepG2mtGFP vaccinated cells, in all cases examined. In addition, both the rate of growth and the size of the tumors were increased in the tumors of HepG2mtGFPGR cells, compared with those of HepG2mtGFP control cells.

In order to investigate the mechanism of action of mtGRin tumor development, the protein levels of molecules involved in autophagy, apoptosis, gluconeogenesis, Krebs cycle and other GR interacting molecules were examined in total and mitochondrial extract from the produced tumors.

Are results showed that in the presence of mtGR, protein levels of enzymes of oxidative phosphorylation and Krebs cycle were deacreased. gluconeogenesis was suppresed as indicated by the decreased level of the gluconeogenic enzyme Phosphoenolpyruvate carboxykinase (PEPCK). This effect resembles the metabolic reprogramming observed upon Warburg effect In addition autophagy was activated and contributed to tumor development, via production of precursors of biomolecules, that are necessary for the generation of the newly synthesized cancer cells. Mitochondrial dependent cells apoptosis was not activated.

In addition, in order to investigate the role of mitochondrial GR, under oxygen availability, the DEX-dependent expression of proteins involved in the mitochondrial function, in HepG2, HepG2mtGFP and HepG2mtGFPGR cells was examined. Our results showed that the regulation of mitochondrial transcription and OXPHOS biosynthesis by mtGR is hormone dependent and followed a diphasic phase upon time of exposure. Whereas, mtGR caused reduction in PEPCK protein levels in all conditions examined. Also, no changes in autophagic proteins were observed, suggesting that the presence of mtGR in mitochondria is not adequate condition for induction of autophagy. In conclusion, mtGR- PDH interaction appears to play a dominant role in the regulation of mitochondrial metabolism. This interaction may lead to suppression of PDH activity, suppression of gluconeogenesis, and induction of metabolic reprogramming, similar to that observed in the Warburg effect. Negative regulation

of mitochondrial transcription by mtGR also support this action. In conclusion, our results contribute to understanding the role of mtGR and discovering new therapeutic targets for treatment of cancer.