Prayogik Rasayan

Glucocorticoid receptor: A potent cellular & gene therapy target for cancer and cancer stem cells

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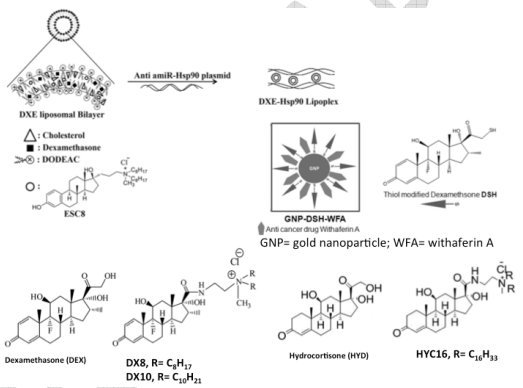
Abstract

Glucocorticoid receptor (GR) is expressed in the cytoplasm of almost all cells, if not all of cancer and non-cancer cells. Moreover, unlike many other factors implicated with cancer, it is neither over-expressed nor is it expressed on cell-membrane surface to qualify logically as a viable target for treating cancer. GR is importantly linked with alternate pathway of energy metabolism in cancer cells and our research indicated that cancer cells possibly tend to avoid activation of GR which would have otherwise instigate that energy demanding alternate pathway called gluconeogenesis. We discovered a way to induce cancer cell-selective GR-transactivation which leads to among many things, gluconeogenesis, reversal of epithelial-to-mesenchymal transition (EMT), drug- sensitization in drug-resistant cancer cells etc. Thus, we proved, although it warrants further studies, that GR in cancer cells behave differently and hence it can be a viable target for the treatment of cancer.



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Introduction

Glucocorticoid receptor (GR) is a nuclear hormone receptor (NHR) that resides in a ligand-unbound state in cytoplasm. Being NHR, as the name suggests, GR upon ligand or hormone (endogenous or synthetic) binding self-activates following its detachment from heat shock protein (HSP90) and goes inside the nucleus, which is called translocation. In nucleus it has multiple roles. Classically, in ligand bound state and in association with multiple other co-transcription factors GR sits on certain promoter regions of our genome called glucocorticoid responsive elements (GRE) and regulates (activates or represses) multiples of GRE-promoted genes. The whole process is called GR-transactivation/transrepression. This process is highly conserved and extents of variation logically depend on cell-types of even different species. This is owing to fact that the ligand-binding domain (LBD) and DNA-binding domain (DBD) are highly homologous even among GRs of different species[1-3]. Hence, non-cancer and cancer cell-expressed GRs are functionally same, ashormone or ligand-mediated GR-recognition followed by GR-transactivation remains same among these cells. This is exemplified by a simple experiment. A fluorescent protein-tagged GR, expressed exogenously in the cytoplasm of a normal and a cancer cell, will recognize the ligand or hormone and translocates itself to nucleus in similar fashion. However, if properly formulated, the GR-ligand can show differential ability of GR-recognition in non-cancer and cancer cells. This short story is about this discovery, which helped us to develop novel therapeutics for cancer.

GR is expressed in almost all cells, cancerous or non-cancerous. The ligands for GR are called glucocorticoids (GCs) and their GR-association leads to multiple functions. Among important functions of GR, maintenance of glucose homeostasis using non-carbohydrate precursors (through a process called gluconeogenesis), regulation of various anti-inflammatory and immunosuppressive actions, protein and fat metabolism etc.are some important functions that relate to various day-to-day pathologic conditions in human being [4,5].

Typically, cancer cells are very proliferative in nature. For its energy need, it mostly, if not fully, depends on glycolysis. The acidic by-products of glycolysis are pyruvic acid and lactic acids, which are in reality, the substrates for gluconeogenesis. Gluconeogenesis process is solely regulated by ligand-bound GR, which by transactivating various GRE-genes produces glucose from non-carbohydrate precursors such as pyruvic acid, lactic acid, glycerol (obtained after fat-hydrolysis), various amino acids etc.Gibbs free energy of glycolysis is -63KJ/mole, whereas, for

gluconeogenesis it is -18 KJ/mole. Understandably, glycolysis is energetically more favourable process than gluconeogenesis and hence energy starved cancer cells depend on glycolysis for quenching its energy demands. The over-dependence on glycolysis leads to accumulation of acidic impurities (lactic acid) in the tumormicroenvironment. How? As lactic acid over-production inside the cancer cell occurs, the acid threatens to change (or acidify) the intracellular pH homeostasis that has the ability to play havoc with the viability of cancer cells itself. The mechanism that sustains cancer cell cannot at the end kill itself. So, cancer cells show the urgency to throw the acidic impurity out by producing various protein pumps to fulfil the immediate demand to restore homeostasis inside the cells. So, as tumor grows bigger and bigger, the expression of these protein pumps [called MDRs, p-glycoproteins etc.] also increases. These pumps are also notorious for developing drug resistance among cancer cells in a tumor lump. So, in this scenario, we hypothesized that if we can forcefully instigate gluconeogenesis in cancer cells we possibly can use up the lactic acid (substrate for gluconeogenesis) and reduce the acid burden in cancer cells. This will reduce the immediate necessity to express these acid-pumping proteins. As a result the drug-resistance should decrease in cancer cells. But the fundamental question is, how to induce gluconeogenesis in the first place?

During 2007-2009, we discovered that GR expressed in cancer cells and non-cancer cells behave quite differently. How we come to this conclusion? We did a simple experiment. We formulated a GC, namely dexamethasone (DEX) in cationic liposomal formulation. Cationic liposomes are well known for their ability to delivery genes to cells toward expressing the corresponding protein (the complex process is called transfection) [6]. Originally we wanted to design a liposomal transfection reagent that would universally transfect all kinds of cells. How can one accomplish that? This is possible by accommodating or associating in liposome such a ligand that targets a receptor ubiquitously expressed in all cells. GR was the first choice of receptor for this design of transfection agent.

Serendipitously, we found that the transfection with this new formulation was not only higher in cancer cells but most importantly the transfection was GR-mediated in only cancer cells. Transfections in non-cancer cells were not GR-mediated, even though these expressed GR. We found that a compromised activity of HSP90 in cancer cells led to this anomalous behaviour of cancer cell-associated GR[7]. Next, we designed an anti-HSP90 gene and delivered it to cancer cells using the same GR-targeted formulation. As the GR-targeted formulation exhibited better transfection in cancer cells compared to that in their non-cancer counterpart, the cancer cells could be transfected efficiently with anti-HSP90 gene and thus reducedtumor burden significantly [8].

To come back to the story about how to combat drug resistance in cancer, we utilized the same GR-targeted formulation to accomplish induction of drug-sensitization in cancer cells. Additionally, cancer stem cells (CSC) are certain very small population in cancer cells that also shows the condition of high drug resistance. CSCs epitomize the advent of the most aggressive and relapsing condition of cancer, as they themselves are drug resistant and they give rise to multiple different clonal populations of cancer cells with excessive aggression in the later stage of cancer. This leads to relapse in cancer. For this condition, there is literally no therapeutics available. As drug resistance is a common phenomenon for advanced stage of cancer and also for CSCs, can't we use the same strategy against CSCs also? Ideally, eliminating CSC is expected to provide therapeutic option for relapsed and advanced stage cancers.

Liposome-based strategy is a wonderful strategy that can accommodate multiple drug cargoes. If the liposome can carry GR-ligand why can't it carry other drugs? Precisely so, we reformulated with individual hydrophobic and hydrophilic drugs in the GR-targeted formulation and treat it to various kinds of very aggressive cancer cells from human pancreatic cancer and melanoma. We also treat these new drug-associated formulations to murine CSCs isolated from breast tumours. We found very efficient drug sensitization in all these kinds of cancer cells [9-10]. In this regard, we would like to bring the readers' attention to another fact that can revolutionize the drug-therapy approaches. We find that using an anti-breast cancer drug co-formulated in this GR-targeted liposome we can kill pancreatic cancer cells also [9]. Clearly, this hints to the potential development of a new platform technology wherein drugs can be repurposed for various other cancer phenotypes, without discovering new drug molecules. The technology is although in its infancy but has shown tremendous potential to develop as a potent, drug-repurposing, multi-modal technology.

We also find that GR-targeting strategy if adapted to gold nanoparticles we find similar observations, i.e., selective drug sensitization and regression of tumour growth[11-12]. GR-targeted polymeric systems also exhibited similar outcome (Data not published). Hence, the versatility of the primary observation is checked in non-liposomal systems also.

Till now our data did not indicate any possibility to develop new small molecule drugs, preferably GR-targeted ones. If cancer cell-associated GR exhibits such a fine selectivity in its action, it is natural to design new GR-targeting anticancer molecules and expect similar anticancer activity. Towards accomplishing this aspect, we chose GR synthetic ligand DEX and natural ligand hydrocortisone (HYD) and modified them by conjugating these with cationic lipids of different chain lengths. The modification was performed in such a way that their GR-targeting ability was not compromised. After a structure activity relationship (SAR) study we find that DEX with cationic lipid of C8 and C10 chain length showed significant anticancer effect. C10 analogue also showed induced drug-sensitization of cancer cells against JAK-STAT pathway inhibitor[13-14]. Surprisingly, not C8 or C10 but only C16 analogue of HYD exhibited selective killing of cancer

cells and significant reduction in tumour aggressiveness in mouse tumour model [15].

The above examples amply prove that GR is indeed a novel target for cancer. Previously, it was not considered as a cancer target as selection of GR defies two common perception of cancer targeting. Firstly, cancer targeting requires over-expression of certain receptor on cancer cells. Normal cells should express the same receptor basally. Secondly, the receptor to target should be preferably expressed on cell surface. GR is neither over-expressed nor is a membrane-bound receptor. Hence, GR was never experimented for the purpose of developing cancer-targeted therapeutics. Our study amply demonstrated that GR is indeed a novel target for cancer. Further research is definitely warranted in future.

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Dr. Rajkumar Banerjee is currently working as Principal Scientist, CSIR-IICT, Hyderabad and Associate Professor of Chemical Sciences, AcSIR, India. His primary research focus is to understand the basis of cancer through the eyes of a chemist. Using chemical biology and bioorganic tools he loves to design new bioactive molecules and delivery systems for targeting, imaging and treating cancer. In the process he has rediscovered, rather reemphasized, new targets in cancer. Notable among these are glucocorticoid receptor, N-end rule pathway etc.