World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:11, No:02, 2017

Toward Understanding the Glucocorticoid Receptor Network in Cancer

Authors : Swati Srivastava, Mattia Lauriola, Yuval Gilad, Adi Kimchi, Yosef Yarden

Abstract : The glucocorticoid receptor (GR) has been proposed to play important, but incompletely understood roles in cancer. Glucocorticoids (GCs) are widely used as co-medication of various carcinomas, due to their ability to reduce the toxicity of chemotherapy. Furthermore, GR antagonism has proven to be a strategy to treat triple negative breast cancer and castration-resistant prostate cancer. These observations suggest differential GR involvement in cancer subtypes. The goal of our study has been to elaborate the current understanding of GR signaling in tumor progression and metastasis. Our study involves two cellular models, non-tumorigenic breast epithelial cells (MCF10A) and Ewing sarcoma cells (CHLA9). In our breast cell model, the results indicated that the GR agonist dexamethasone inhibits EGF-induced mammary cell migration, and this effect was blocked when cells were stimulated with a GR antagonist, namely RU486. Microarray analysis for gene expression revealed that the mechanism underlying inhibition involves dexamenthasone-mediated repression of well-known activators of EGFR signaling, alongside with enhancement of several EGFR's negative feedback loops. Because GR mainly acts primarily through composite response elements (GREs), or via a tethering mechanism, our next aim has been to find the transcription factors (TFs) which can interact with GR in MCF10A cells.The TF-binding motif overrepresented at the promoter of dexamethasone-regulated genes was predicted by using bioinformatics. To validate the prediction, we performed high-throughput Protein Complementation Assays (PCA). For this, we utilized the Gaussia Luciferase PCA strategy, which enabled analysis of protein-protein interactions between GR and predicted TFs of mammary cells. A library comprising both nuclear receptors (estrogen receptor, mineralocorticoid receptor, GR) and TFs was fused to fragments of GLuc, namely GLuc(1)-X, X-GLuc(1), and X-GLuc(2), where GLuc(1) and GLuc(2) correspond to the N-terminal and C-terminal fragments of the luciferase gene.The resulting library was screened, in human embryonic kidney 293T (HEK293T) cells, for all possible interactions between nuclear receptors and TFs. By screening all of the combinations between TFs and nuclear receptors, we identified several positive interactions, which were strengthened in response to dexamethasone and abolished in response to RU486. Furthermore, the interactions between GR and the candidate TFs were validated by co-immunoprecipitation in MCF10A and in CHLA9 cells. Currently, the roles played by the uncovered interactions are being evaluated in various cellular processes, such as cellular proliferation, migration, and invasion. In conclusion, our assay provides an unbiased network analysis between nuclear receptors and other TFs, which can lead to important insights into transcriptional regulation by nuclear receptors in various diseases, in this case of cancer.

Keywords : epidermal growth factor, glucocorticoid receptor, protein complementation assay, transcription factor
Conference Title : ICNRD 2017 : International Conference on Nuclear Receptors and Diseases
Conference Location : London, United Kingdom
Conference Dates : February 16-17, 2017