55 GPRC5A facilitates cell proliferation and bone metastasis of prostate cancer

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Introduction & Objectives: The prognosis of the patients with hormone refractory and/or metastatic prostate cancer is poor. To overcome this disease, the investigation of novel therapeutic target molecule has been desired. Among various therapeutic target molecules, orphan GPCRs have high potential to treat diseases. Therefore, this study is aimed to identify novel therapeutic target molecules for progressive prostate cancer using integrative genome-wide gene expression analyses.

Materials & Methods: Microarray data sets of these cell lines, such as LNCaP, DU145 and PC3, were obtained from Gene expression omnibus in NCBI (PC3: n = 9, DU145: n = 6, LNCaP: n = 33) to identify the genes, which expression is remarkably higher in DU145 and PC3 than LNCaP among orphan GPCR genes. Knockout (KO) of target genes in PC3 and DU145 cells were generated by CRISPR/Cas9, and the cell proliferation ability was analyzed by BrdU incorporation. To analyze the significance of the gene in vivo, subcutaneous xenograft mice models were analyzed by in vivo imaging and histological examinations. To explore the gene expressions profiles, RNA-seq, was performed. Also, to ascertain the effects of target gene on the establishment of bone metastasis of prostate cancer, Control and KO PC3-Luc cells were inoculated into the tibia, which were analyses by in vivo imaging, radiological and histological examinations. Kaplan meier plot (KMP) analysis was done using RNA-seq data from The Cancer Genome Atlas project.

Results: Integrative genome-wide analyses successfully identified G Protein-Coupled Receptor Class C Group 5 Member A (GPRC5A) as the target gene. GPRC5A KO PC3 cells exhibited significantly decreased cell proliferation in vitro. In addition, subcutaneous xenograft model displayed that the size and weight of removed tumors were remarkably decreased in GPRC5A KO PC3 cells when compared to Control cells. RNA-seq revealed that the expression of cell cycle related genes were significantly impaired in GPRC5A KO cells. Consistent with this result, the expression of both G2 and M phase genes were significantly elevated and G1 phase genes were significantly decreased by GPRC5A KO. In addition, flow cytometry analyses revealed that GPRC5A KO cells were arrested in the G2/M phase. Furthermore, the expression level of GPRC5A in prostate cancer with bone metastasis is significantly higher in that without bone metastasis. Also, the mice inoculated with Control PC3 cells demonstrated bone destructive metastatic lesions in the proximal tibia, however, GPRC5A KO PC3 cells failed to establish bone metastasis. Finally, KMP analysis showed that patients with high expression of GPRC5A had significantly shorter overall survival.

Conclusions: GPRC5A is essential for cell proliferation and establishment of bone metastasis of prostate cancer, suggesting GPRC5A can be a therapeutic target and prognostic marker molecule for progressive prostate cancer.