RESEARCH ARTICLE

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The Correlation between YAP and RhoA Expression in Prostate and Ovarian Tumor Stroma

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Abstract

Background and objective: Cancer associated fibroblasts (CAFs) are a mesenchymal cell type found in most solid tumors modulating cancer metastasis by building up and remodeling the extracellular matrix (ECM) structure. We aimed to evaluate the correlation between *RhoA* and *YAP* expression in the stroma cells obtained from prostate and ovarian cancer tissues. **Methods:** We analyzed two microarray datasets obtained from NCBI Gene Expression Omnibus(GEO). The sample type of two datasets was RNA, which is displaying the transcriptome profiling. The tumor stroma of patients with invasive prostate cancer and high-grade serous ovarian cancer were obtained from datasets Independent t-test was used to analyze the differentially expressed *YAP* between normal stroma and cancer stroma. In addition, Pearson's correlation was run to analyze the correlation between *YAP* and *RhoA* expressions. **Results:** In comparison with normal stroma tissues, *YAP1* was overexpressed in prostate and ovarian cancer stroma tissues (prostate cancer stroma, $p < 0.05$; ovarian cancer stroma, $p < 0.001$). Furthermore, a positive correlation was detected between *YAP* and *RhoA* expressions in stroma of both tumor types. This correlation was positively strong in prostate cancer stroma ($R=0.607$) and positively weak in ovarian cancer stroma ($R=0.248$). **Conclusion:** We found that *YAP* was overexpressed in prostate and ovarian cancer stroma. Furthermore, the correlation between *RhoA* and *YAP* expression suggested that *RhoA*-*YAP* signals could physiologically be involved in tumor stroma. Thus, targeting *RhoA*-*YAP* may be an intriguing avenue for cancer therapeutics in neoplastic epithelial cells as well as tumor stroma.

Keywords: Tumor microenvironment- cancer-associated fibroblasts- YAP- RhoA

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Introduction

Over the past decade, the tumor microenvironment (TME) has come to be recognized as an initiator and a promoter of tumorigenesis. Cancer-associated fibroblasts (CAFs) are one of the most dominant components in the TME (Kalluri and Zeisberg, 2006). CAFs are a mesenchymal cells found in most solid tumors modulating cancer metastasis by restructuring and enhancing the extracellular matrix (ECM) (Erdogan and Webb, 2017). Furthermore, CAFs produce growth factors, stimulate tumor angiogenesis, modulate drug access, and respond to cancer therapy (Sahai et al., 2020).

We previously studied the mechanical characteristics and roles of CAFs in cancer progression and found that CAFs led to Ras homolog gene family member A (*RhoA*) overexpression-mediated cytoskeletal alteration and *YAP* (Yes-associated protein) activity, thereby enhancing aggressive phenotypes, including invasiveness and migratory capacity (Kim et al., 2019). Our previous study focused solely on CAFs adjacent to oral squamous cell carcinoma (OSCC). However, most tumors share certain molecular signatures related to cancer, including the sustainment of proliferative signals, evasion of growth suppressors, cell death resistance, angiogenesis induction, and activation of invasion and metastasis (Yuan et al., 2016). For example, the overexpression of HER2/neu (ERBB2) has been observed in breast cancer, salivary gland tumors, and uterine and gastric cancers (Oh and Bang, 2020). In addition, Wingless-Int (WNT) and transforming growth factor beta (TGF-beta) play critical roles in the regulation of malignant phenotypes in various cancer types (Massague, 2008; Anastas and Moon, 2013). Moreover, specific somatic mutations, such as *TP53* and *KRAS* mutations, can be exhibited across a wide variety of tumor tissues, including lung and pancreas tumors (Schneider et al., 2017). Hence, we aimed to investigate whether the correlation between *YAP* and *RhoA*, which we previously identified in fibroblasts surrounding OSCCs, could be observed in the stroma surrounding different tumor types.

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Materials and Methods

NCBI Gene Expression Omnibus (GEO) dataset analysis

We used the publically available data sets in our study downloading the array data for GSE26910 and GSE40595 from NCBI Gene Expression Omnibus(GEO) (http://www.ncbi.nlm.nih/gov/geo/) database with the platform of the GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array, which were deposited by some researchers (Planche et al., 2011; Yeung et al., 2013). The sample type was RNA, which are displaying the transcriptome expression profiling . The database of GSE26910 contains 24 samples, namely 6 stroma samples surrounding invasive breast primary tumors, 6 matched samples of normal stroma, 6 stroma samples surrounding invasive prostate primary tumors, and 6 matched samples of normal stroma. Among them, we selected 12 samples of prostate cancer stroma and normal prostate stroma. The database of GSE40595 contains 77 samples, including 31 microdissected cancer associated stroma samples, 32 epithelial tumor samples from high grade serous ovarian cancer patients, 8 microdissected normal ovarian stroma samples, and 6 ovarian surface epithelium samples. We selected 39 samples from normal stroma samples and cancer associated stroma samples.

Statistical analysis

All statistical analyses were done by using SPSS version 20 (SPSS Inc., Chicago, IL, USA). For GEO datasets analysis, independent t-test was used to analyze the difference in specific gene expression between normal stroma and cancer stroma. In addition, Pearson's correlation coefficient was used to analyze the correlation between specific genes.

Results

Difference between normal stroma and tumor stroma regarding YAP expression

We first investigated differentially expressed *YAP* gene between normal stroma and tumor stroma by using publically available data sets from NCBI. We selected two data sets. The first data set included prostate tumor stroma and normal stroma of patients with invasive prostate cancer (n=6). The second data set involved ovarian cancer stroma (n=31) of high-grade serous ovarian cancer patients and normal stroma of healthy individuals(n=8). All intensity values were normalized to log base 2 values. In comparison with normal stroma of prostate and ovarian cancer, the overexpression of *YAP*1 was observed in both cancer stroma tissues (Figure 1A and 1B).

The correlation between YAP and RhoA expression in tumor stroma

RhoA is upstream of the *YAP* transcriptional factor and contributes to *YAP* distribution in CAFs surrounding OSCC cells, suggesting that *YAP* expression is correlated with *RhoA* expression. To verify this correlation in stroma surrounding other tumor types, we analyzed the correlation between *RhoA* and *YAP* expressions in prostate and ovarian cancer stroma. The positive correlation between *YAP* and *RhoA* was identified in the stroma of both tumor types (Figure 2A and 2B). Prostate cancer stroma showed a strong positive correlation (R=0.607) (Figure 2A), and ovarian cancer stroma showed a weak positive correlation (R=0.248) (Figure 2B).

Discussion

Cancer studies have primarily focused on neoplastic epithelial cells (Dagogo-Jack and Shaw, 2018). However, increasing evidence suggests that the TME plays a critical role in tumor initiation, progression, and therapeutic resistance (Sun, 2016). CAFs constitute a large proportion of the TME; therefore, CAF-targeted cancer therapies are extensively being explored. For CAF-targeting strategies, several CAF markers, such as smooth muscle actin (α-SMA), fibroblast activation protein (FAP), caveolin-1 (cav-1), and platelet-derived growth factor (PDGF), have been studied in the past years (Chen and Song,

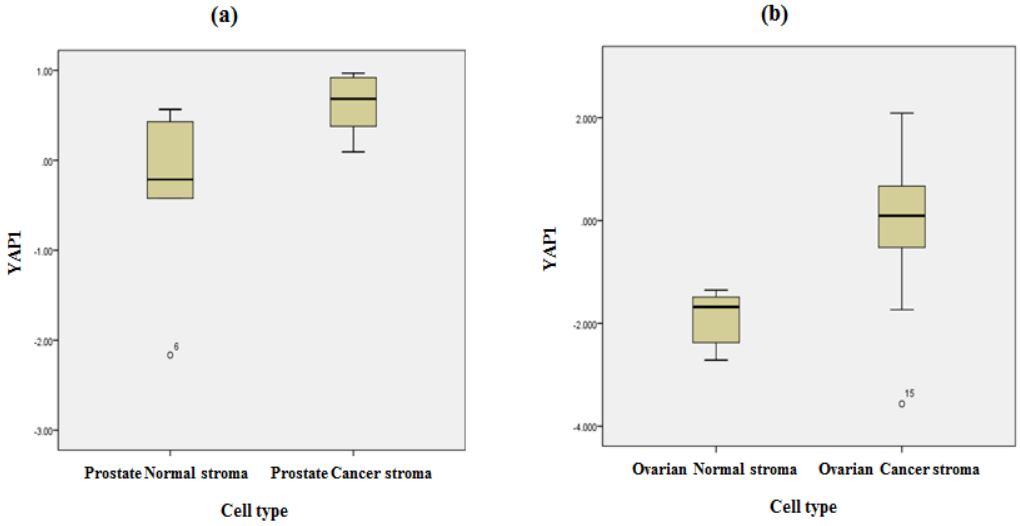

Figure 1. *YAP* Expression in Cancer Stroma Obtained from Prostate and Ovarian Patients. a, The samples were acquired from prostate cancer stroma and normal stroma in GDS4114 (*YAP1*, *p=0.047); b, The samples were acquired from ovarian cancer stroma and normal stroma in GSE40595. (*YAP1*, ***p=0.000)

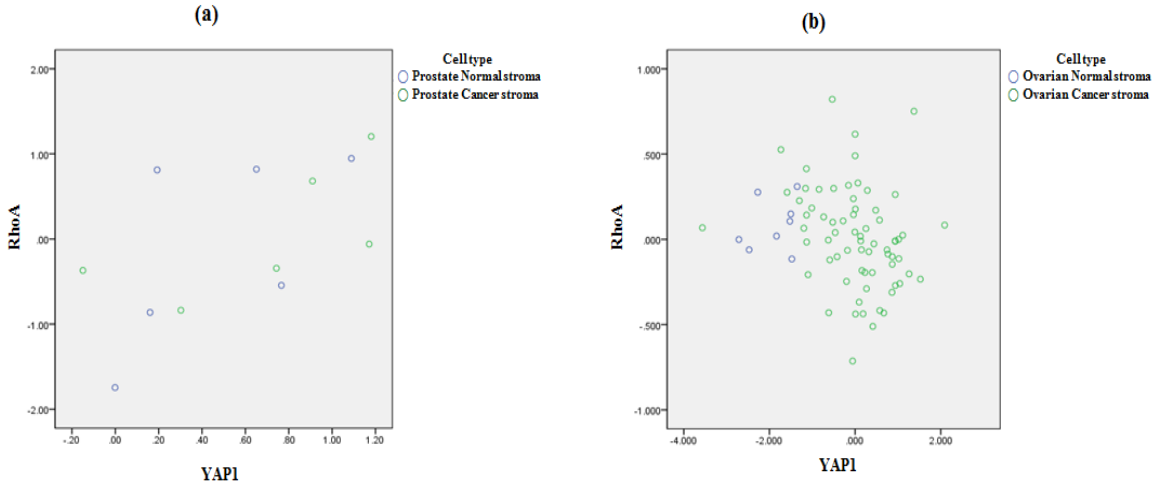

Figure 2. The Correlation between *YAP* and *RhoA* Expression in Normal Stroma and Cancer Stroma. a, The correlation between *YAP* and *RhoA* in normal stroma and prostate cancer stroma (Pearson correlation, R=0.067 (strong positive (+) correlation), *p=0.036; b, The correlation between *YAP* and *RhoA* in normal stroma and ovarian cancer stroma in GSE40595 (Pearson correlation, R=0.248 (weak positive (+) correlation), *p=0.029).

2019; Biffi and Tuveson, 2021). However, ubiquitous CAF-targeting agents remain unclear because CAFs have heterogeneous subtypes. CAFs can be derived from numerous cellular sources adjacent to cancer cells, including normal fibroblasts, bone marrow stem cells, and mesenchymal cells transited from epithelial cells (epithelial-mesenchymal transition; EMT) (Chen and Song, 2019; Mhaidly and Mechta-Grigoriou, 2020). The precise origin of CAFs has not yet fully elucidated, implying that the heterogeneity of CAFs is rooted in the diverse cellular subsets. Moreover, CAFs have the phenotypical heterogeneity that activated and senescent phenotypes. Myofibroblasts are considered to be a subset of activated fibroblasts that have contractile and secretory profiles such as alpha-1 type I collagen (COL1A1), interleukin (IL)-1 family member (IL-1α), IL-6 α, and C-X-C Motif Chemokine Ligand 1 (CXCL1)/growth-regulated oncogene (GRO) -α, contributing to tissue repair during wound healing and cancer development (Kalluri and Zeisberg, 2006; Bae et al., 2014). Conversely, the senescent phenotype of CAFs is observed adjacent to cancer cells and can secret a senescence-associated secretory protein (SASP) and exacerbate cancer progression, including cancer cell invasion and migration (Wang et al., 2017; Kim et al., 2018a). In addition to phenotypic heterogeneity, functional heterogeneity of CAFs has also been reported. CAFs have historically been considered as tumor-promoting components that enhance cancer cell proliferation, angiogenesis, and metastasis (Erez et al., 2010; Benyahia et al., 2017). However, some studies have been reported that CAFs might have tumor-restraining functions (Biffi and Tuveson, 2021). Stroma cells can reduce neoplastic epithelial cell growth through Hedgehog pathway activity in pancreatic ductal adenocarcinoma (Lee et al., 2014; Rhim et al., 2014). Moreover, Meflin, which is glycosylphosphatidyl inositol (GPI)-anchored protein, suppresses tumor growth in CAFs (Mizutani et al., 2019). As CAFs have the molecular, phenotypic, and functional heterogeneity, targeting specific CAFs is the most challenging aspect. Thus, we previously focused on the common physical characteristics of CAFs, regardless of their diverse cellular origin and their functional and phenotypic heterogeneity. CAFs have traditionally shown higher contractility through ECM remodeling. Moreover, this contractility has been previously reported to aid differentiation between CAFs and normal fibroblasts (Kim et al., 2019; Nurmik et al., 2020). Thus, our findings suggested that more specific markers for tumor stroma may be associated with the physical characteristic of CAFs, *YAP* overexpression, and *RhoA* expression .

YAP and *TAZ* (transcriptional co-activator with WW and PDZ domains; WWTR1) interact with TEAD-family DNA-binding transcription factors. Their interaction influences tissue homeostasis, regeneration, and organ size, and can be responded to Hippo signaling, Wnt, and G-protein coupled receptors (GRCR) (Zanconato et al., 2016) . In a wide variety of human cancers, *YAP* is thought to be a major contributor to tumorigenesis (Zanconato et al., 2016; Thompson, 2020). For example, *YAP* overexpression is associated with poor clinical outcomes in ovarian cancer (He et al., 2015a), cervical cancer (He et al., 2015b), and liver cancer (Liu et al., 2010). Moreover, *YAP*/*TAZ* functions as an executer by regulating ECM elasticity, cell shape, and cytoskeletal forces, thereby remodeling the metastatic environment in cancer cells (Dupont et al., 2011; Yu et al., 2015). Consistent with these findings, CAFs showed that *YAP* expression was regulated by the conformation and tension of the actin cytoskeleton, as the downstream effector of Rho GTPase pathways (Aragona et al., 2013; Calvo et al., 2013; Kim et al., 2019). In a nutshell, these results indicated that *YAP* was a core factor in the mechanical regulation of both neoplastic epithelial cells and stroma cells. Furthermore, *RhoA*, which is a member of the Rho GTPase subfamily proteins, including *RhoA, Cdc42,* and *Rac1/2*, is required for *YAP*/*TAZ* activation according to a previous study (Kim et al., 2018b). Rho GTPases are well known in diverse biological processes, including cytoskeleton regulation, cell adhesion, and cell migration (Haga and Ridley, 2016).

Among the Rho GTPases, *RhoA* is involved in tumor progression and metastasis. The overexpression and activity of *RhoA* are observed in many malignant tumors, such as bladder cancer, liver cancer, and gastric tumors (Gomez del Pulgar et al., 2005). Additionally, *RhoA* can influence the cell cycle checkpoint and regulate cancer cell survival in gastric cancer cells (Zhang et al., 2009). Thus, *RhoA* has been considered as a promising target for anti-cancer therapy.

Our study revealed that *RhoA-YAP* could be a promising therapeutic target. However, our study faced with some limitations. One major limitation was that we focused on *YAP* expression, not *TAZ* expression. However, *TAZ* is a paralog co-activator of *YAP* and functions as a downstream effector of the mechanical signaling via the RhoA-ROCK pathway, indicating that *TAZ* along with *YAP* could be crucial cancer therapy target. Moreover, we investigated the expression and correlation between *YAP* and RhoA in two tumor types, namely prostate and ovarian cancers. To generalize the ubiquitous CAFs markers from a physical point of view, further studies are needed to investigate this gene expression and its correlation with tumor stroma surrounding diverse tumor types. We found that *YAP* was overexpressed in the stroma adjacent to different tumor types, such as prostate and ovarian cancers, as well as OSCC. A correlation between *RhoA* and *YAP* wasalso been observed in both cancers. Thus, targeting *RhoA-YAP* signals in CAFs and neoplastic epithelium might be a comprehensive therapeutic approach in prostate and ovarian cancers aw well as OSCC, restricting the formation of a tumor-promoting microenvironment.

Author Contribution Statement

Conceptualization: D.K. and M.-J.L.; Design: M.-J.L.; Literature search: D.K.; Data acquisition: D.K.; Data analysis: M.-J.L.; Manuscript preparation: D.K.; Manuscript editing: D.K. and M.-J.L. All authors reviewed the results approved the final version of the manuscript.

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I mistook the this research is part of a PhD thesis (Dokyeong Kim) approved by Yonsei. However, I identified that the results are not involved in my PhD thesis.

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Any conflict of interest

The authors have no conflict of interest to declare.

Ethical issues

Not applicable. The data for this study was obtained from public database registered in NCBI.

Availability of data

All datasets leading to the results of the study are available by the corresponding author on reasonable request.

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