Expression Of Hippo-YAP Signaling Pathway in Gynecological Tumors

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Abstract. Ovarian cancer, breast cancer and cervical cancer are common female malignant tumors. YAP signal is widely activated in human malignant tumors. Hippo-YAP signal pathway affects the proliferation and development of tumors. However, the transmission mechanism of YAP signal in gynecological malignant tumors is still unclear. The function of YAP in the Hippo signal route is examined in this research, along with its functions upstream, middle, and downstream of the Hippo signal route. In addition, the mRNA expression data of YAP in ovarian serous cystadenocarcinoma and normal ovarian tissue is compared. The expression of YAP in breast invasive carcinoma (BRCA) is observed by IHC, and the YAP intensity of cytoplasm and nucleus is evaluated. Through the relationship between YAP and HPV, to further investigate the mechanism of YAP in cervical cancer, the distribution of YAP1 in cells adhering to the cervical surface was studied. These findings are critical in the treatment of YAP as a potential therapeutic target. Furthermore, understanding the role of YAP in female malignant tumors provide an insight in developing drugs that block the activity of YAP or inhibit the activation of YAP, they can play an anti-tumor role in vivo.

Keywords: YAP, Hippo signaling pathway, TEAD, Gynecological tumor, Therapeutic target.

1. Introduction

Yes-associated protein (YAP) is essentially a proline-rich phosphoprotein that is a multifunctional intracellular linker and transcriptional coactivator encoded by the YAP1 gene. Because to its 65 ku molecular weight, it is also known as YAP65. YAP can be used as the main effector of Hippo tumor suppressor gene pathway. YAP gene, located in chromosome 11q22 region, is considered to be a proto oncogene. Its encoded product is YAP protein, which widely exists in various tissues of the body. As shown in Figure 1, the protein comprises a number of fundamental structural components, including two WW protein domains, PDZ binding sites, helical domains for transcription control, and binding sites for the 14-3-3 phosphorylation.

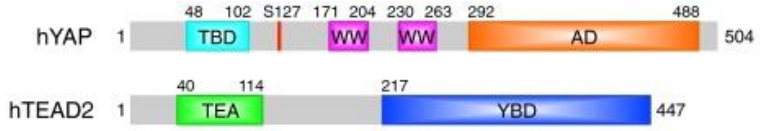

Figure 1. Organization diagram of human YAP and TEAD2 protein domain TBD: TEAD binding domain; S127: Serine 127 (an important binding site related to phosphorylation) AD: an activation domain for transcription; TEA: TEA domain that binds to DNA; YBD: domain that binds YAP [1]

YAP is the main core response factor within the Hippo cascade reaction, which help to control organism growth. Therefore, YAP can regulate the activity of stem cells to a certain extent by acting on various kinases in the Hippo pathway, and then affect the growth and development of tissues and organs in the body through cell division and differentiation.

In modern society, the most common malignancies of female reproductive system are endometrial carcinoma, cervical carcinoma and ovarian carcinoma. Ovarian or cervical cancer affects more women in China, which seriously affect the health of women. Among them, the most frequently observed kind of ovarian epithelial malignant tumor is serous tumor of the ovary. serous malignant tumors account for 50% of ovarian cancer, with a high mortality rate, which can be divided into two types: high-level and low-level. BRCA gene mutation plays a key role, and other homologous

recombination repair genes explain 50% of high-level serous cystadenocarcinoma. YAP1 is an oncogene and overexpressed in ovarian cancer. Breast cancer is also a common gynecological malignancy, accounting for a quarter of the incidence rate of all cancer types in the world, and accounting for about 15% of the female mortality rate. In clinical practice, it needs to be identified by immunohistochemical testing, Routine tests include ER (estrogen receptor), PR (progesterone receptor), etc. ER and PR are collectively called hormone receptors (HR). Generally, they form different combinations and distinguish different breast cancer subtypes according to their positive and negative expressions. Cervical cancer is also one of the four most common cancers for women. However, in developed countries, thanks to HPV vaccination, the incidence rate of cervical cancer is very low, and about 84% of cervical cancer occurs in developing countries. HPV16/18 infection accounted for 70%, belongs to the super high-risk type, there are some HPV and condyloma, generally not malignant change. HPV has E6 and E7 genome encoding carcinogenic proteins, they can inhibit the carcinogenic proteins in the human body, so that the cells infected by HPV infinite proliferation and malignant transformation, the development of cervical cancer. SWI/SNF is an important chromatin remodeling complex, of which ARID1A is the core subunit and a tumor suppressor gene with a high mutation rate in cancer cells. ARID1A mutations are common in cervical cancer patients. YAP is associated with ARID1A through SWI/SNF, providing ideas for the therapeutic targets of cervical cancer [2].

2. Hippo-YAP signal pathway

2.1. Upstream regulations of Hippo-YAP signal pathway

The core of Hippo signaling pathway is regulated by many upstream signaling factors, most of which come from the cell membrane [3]. Many intracellular physiological processes regulate the Hippo signaling pathway, acting as upstream components. For example, due to cell adhesion, Hippo signal pathway responds to external mechanical forces, and acts on RHO kinase in various physiological activities generated by cell and cell extracellular matrix (ECM) adhesion, and then affects actin phosphorylation LATS1/2 (Figure 2). Therefore, Hippo signal pathway constitutes a sensor to ensure the integrity of tissues and cells, rather than specifically reacting to specific extracellular signal molecules. At this stage, studies [4] show that changes in ECM and cell surface receptors are accompanied by changes in nuclear localization, phosphorylation and activity of YAP, and that actin can regulate YAP. In addition, such as receptor tyrosinase (RTKs) and G protein coupled receptors (GPCRS) (Figure 2), which are also similar mechanisms through acting on other key proteins to mobilize the core of Hippo signaling pathway, namely, the kinase cascade reaction of YAP. Adhesion and tight connection occupy an important position in Hippo signal pathway. For example, the complex of Crumbs and aPKC is directly connected to the core of Hippo signal pathway through the angiopoietin (AMOT) protein family, α- Catenin binds to the 14-3-3 binding site of YAP protein, and E-cadherin (E-cadherin expression deletion is a marker of epithelial mesenchymal transformation (EMT), which is related to the increased risk of cancer metastasis) forms a complex with it to activate a cascade of phosphorylation. As well as cooperation with WNT signal path, the inactivation of the destruction complex after stimulation of trcp mediated degradation WNT will also drive β-Catenin YAP/TAZ nuclear translocation.

2.2. The role of YAP in the middle and lower reaches of Hippo signal pathway

YAP is a central regulatory protein in the middle and lower reaches of Hippo signal pathway [5]. Under normal activation, YAP is phosphorylated by Lats1 and Lats2 (kinases), which are phosphorylated by MST1/2 assisted by upstream SAV1, at site 127 (S127) of serine. The phosphorylated binding site on YAP can bind to 14-3-3 protein (14-3-3 protein is a kind of small molecule protein widely existing in eukaryotic organisms, which itself lacks protease activity, but it can achieve its physiological function by binding with target protein and regulating target protein. 14-3-3 protein regulates many physiological activities, such as cell signal transduction, apoptosis, cell

cycle, cell metabolism and cell invasion). The YAP is limited to be degraded and inactivated in the cytoplasm，unable to enter the nucleus. When the Hippo pathway is affected by some factors, the biological activity within the pathway is lost and cannot be activated, the step-by-step phosphorylation reaction cannot be activated. The unphosphorylated YAP escapes from the bondage and enters the nucleus. It combines with the transcriptional coactivator transcription enhancement domain protein TEAD to promote cancer cells to play the role of cancer stem cells, start the DNA replication program, and trigger tumor proliferation, progress and metastasis. It should be noted that whether YAP is phosphorylated or not, it can strongly interact with a variety of tight junctions and/or adhesive junction proteins, which also limits the entry of YAP into the nucleus to affect cell proliferation and differentiation. At present, high expression of YAP is also found in many gynecological tumors.

YAP, as a major transcriptional coactivator downstream of the signaling pathway, regulates cell growth and death by a number of events. When Hippo signaling pathway is inhibited, YAP can be activated and enter the nucleus for transcription and the transcriptional activity of target genes CTGF and AREG increases, further leading tumor formation. On the contrary, When the Hippo signaling pathway is triggered, YAP is phosphorylated and remains in the cytoplasm, inactivating its activity. YAP gene activation promotes the incidence and progression of various cancers, but the significance of YAP expression level in different tumors is different.

YAP has the function of regulating cell proliferation and differentiation, organ growth and regeneration, and accelerating tumor formation. The activation of YAP can cause uncontrolled inhibition of proliferation and apoptosis, leading to the generation of malignant tumors. Under normal circumstances, YAP protein is strictly controlled.

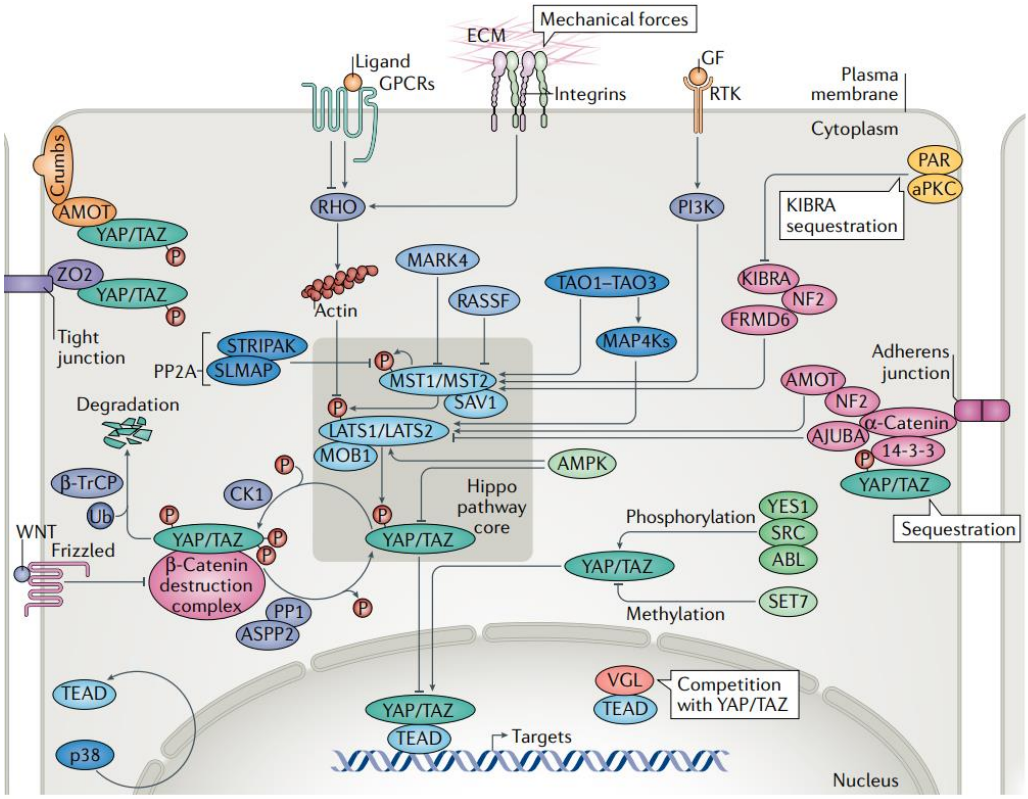

Figure 2. The composition and regulation of Hippo signal pathway. The diagram shows some components of Hippo signal pathway. This schematic diagram highlights the main aspects of Hippo signal pathway, including yeast Ste20 kinase MST1 and MST2, large tumor inhibitory kinase LATS1 and LATS2, their cofactors SAV1, MOB1A and MOB1B transcriptional coactivators, the transcriptional coactivators of YAP and TAZ, and the transcriptional factors TEAD1-TEAD4 family. This core part is that the kinase MST1/2 phosphorylates LATS1/2 kinase, thereby phosphorylating YAP/TAZ protein [3]

3. YAP1 in ovarian cancer

Cho [6] et al. discovered that YAP was found in substantially higher concentrations in tumors than in normal tissues (Figure 3A). Additionally, it was discovered that YAP1 production was greater in the late stage compared to the early stage (Figure 3B), and it was mainly located in the nucleus (Figure 4A), indicating that overexpression of YAP in Hippo YAP/TAZ pathway was implicated in the emergence and progression of ovarian cancer. Many ovarian cancers originate from fallopian tubes, and many evidences tend to prove that high-level serous ovarian cancers originate from the secretory epithelial cells of fallopian tubes [7-9]. Studies have shown that YAP overexpression in inflammatory and cancerous oviduct tissues can lead to tumor-forming malignant cell proliferation, so create a positive feedback loop with the Hippo route and certain pathways that promote endothelial cell development, further promoting the progress of cancer [10]. Therefore, the combination of YAP inhibitor and fibroblast growth factor receptor inhibitor can provide a new therapeutic strategy for high-grade serous ovarian cancer.

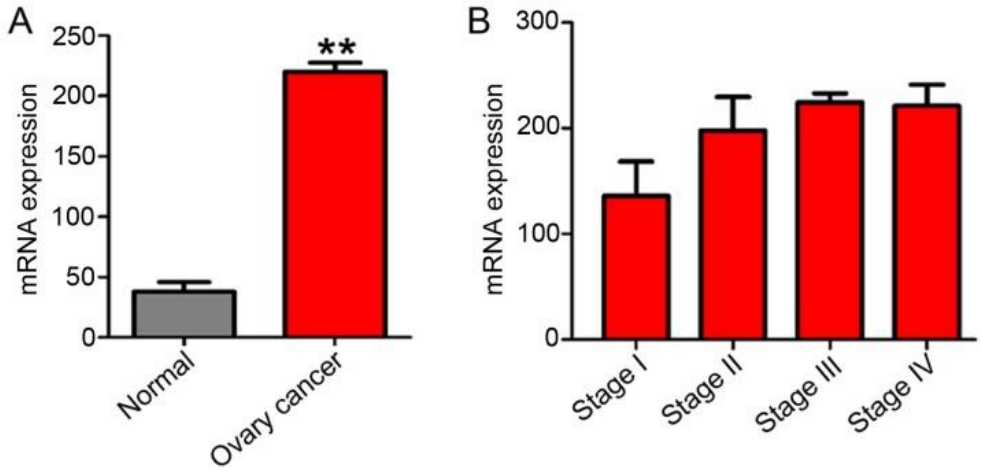

Figure 3. Expression of YAP1 mRNA in serous tumor of ovary. (A) YAP1 mRNA data from a database of normal controls and serous ovarian cystadenocarcinoma. (B) mRNA expression of YAP1 in different stages of ovarian serous cystadenocarcinoma [6]

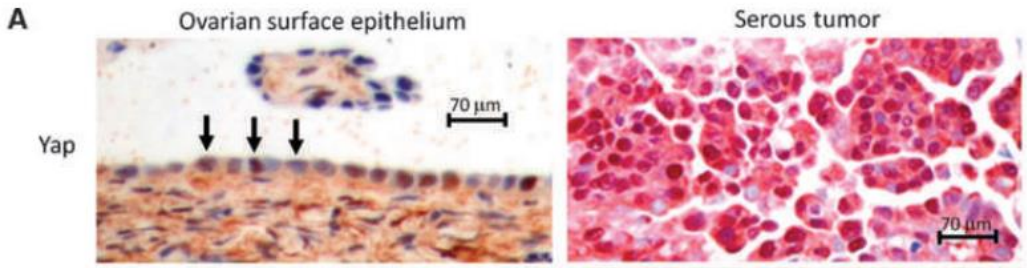

Figure 4. (A) Ovarian epithelial cells (left) and serous tumors (right) use hematoxylin (blue) to show the nucleus, as well as YAP (brown in ovarian epithelial cells and red in serous tumors). The arrow points to the main localization and nucleus of YAP [11].

The study found that the positive expression rate of YAP in epithelial ovarian cancer was 90.57%, while it was only 50% and 65% in normal ovarian tissue and benign epithelial ovarian tumors, which shows a statistically significant difference. YAP protein is involved in accelerating cell proliferation, resisting cisplatin induced apoptosis, accelerating cell migration and anchoring independent growth [12].

It has been proved that YAP can promote the invasion and metastasis of ovarian cancer. Studies on cellular levels have found that YAP is related to many characteristics that promote tumor invasion and metastasis, such as the disappearance of cell contact inhibition, anchoring independent growth, and epithelial mesenchymal transition (EMT). Zhang et al. [13] pointed out that when tumor cells are fully grown, the total number of cells reaches a stable state and begins to apoptosis, while ovarian

cancer cells activated by YAP overexpression can continue to proliferate after fully growing, indicating that cells have lost contact inhibition.

4. YAP in breast cancer

Li et al. [14] showed that YAP signal had a carcinogenic effect in malignant breast tumors. In contrast to the normal tissue in the control group (more than 5cm from the edge of the cancer tissue), YAP expression rose in the breast cancer group. Moreover, individuals with lymph node metastases had considerably higher expression rates of YAP. In addition, Eva [15] et al. studied about 200 human breast cancer samples, the position of YAP expression in cells and the symptoms of breast tumor were observed. To investigate the mechanism of expression of YAP in BRCA, the sample tissues were stained (Figure 5). Because the function of YAP can shuttle outside the cytoplasm and nucleus, the strength of YAP was evaluated in both cytoplasm and nucleus. It is concluded that the increase of YAP in cytoplasm is related to the tumor differentiation of YAP and ILC in nucleus. In addition, the level of YAP found in the nucleus of e-cadherin ILC is higher, so the change of YAP in the nucleus of breast tumor can be used to determine the ILC. Researchers [16] investigated by identifying breast cancer cell lines related with the Hippo pathway and monitoring YAP/TAZ activity in each stage of breast cancer. YAP/TAZ activity was significantly increased in low differentiation (high grade) G3 of breast cancer. The above study revealed that Hippo cascade was activated in cell-cell Scribble in non transformed and tumorigenic breast epithelium. Scribble is a connector that can assemble protein complexes with TAZ, LATS and MST. Scribble is required for activation of Hippo cascade phosphorylation.

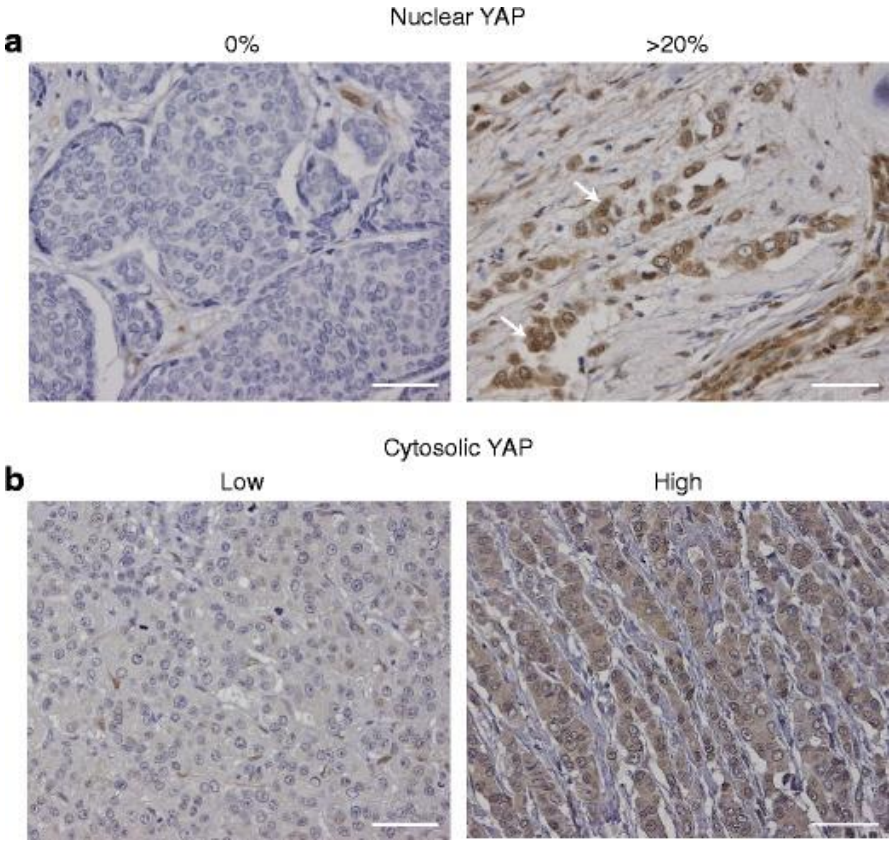

Figure 5. YAP expression levels shown in human breast invasive carcinoma with IHC. A The manner in which YAP is expressed in the nucleus. The white arrow points to the stained part of the nucleus. The proportion of YAP expression in the nucleus, with a score of 0% (left figure) and more than 20% (right figure). B The expression level of YAP in cytoplasm. The cytoplasmic YAP expression score is low YAP (left figure) or high YAP (right figure). The sample shown in the right figure also obtained a score of<20% (15-20%) in nuclear YAP positioning [15].

5. YAP1 in cervical cancer

About 70% of cervical cancer is related to HPV16 and 19, and about 98% of cervical cancer in China is caused by high-risk HPV infection. In the case of overexpression of YAP, the effect of immune factors in the body will be severely weakened, so that the immune system of the human body will be damaged, leading to the increase of HPV infection rate. The mouse model [17] showed that overexpression of YAP induced the growth of malignant cervical squamous cell tumors within about 7 months. In addition, using one group of mice with HPV oncogene and induced YAP overexpression and another group of mice with YAP overexpression, controlling for other variables, it was found that malignant tumor formation was reduced to four months. Studies have shown that over activated YAP1 promotes cervical epithelial cells to be infected by HPV by up regulating the manifestation of HPV receptors and decreasing the body's ability to express immunological factors. On the contrary, YAP1 and HPV work together enhance the sustainability of invasive cervical cancers. HPV can enter the epithelial basal tissue through small skin damage (Figure 6A). YAP1 was shown to be significantly expressed in humans and mice cervical stroma by IHC. According to these findings, from damage to recovery, basal cell basal YAP encourages HPV infection. By cultured and injured human cervical epithelial cells, large amounts of YAP1 were found in the nucleus of the damaged area, but both inside and outside the nucleus of the undamaged area.

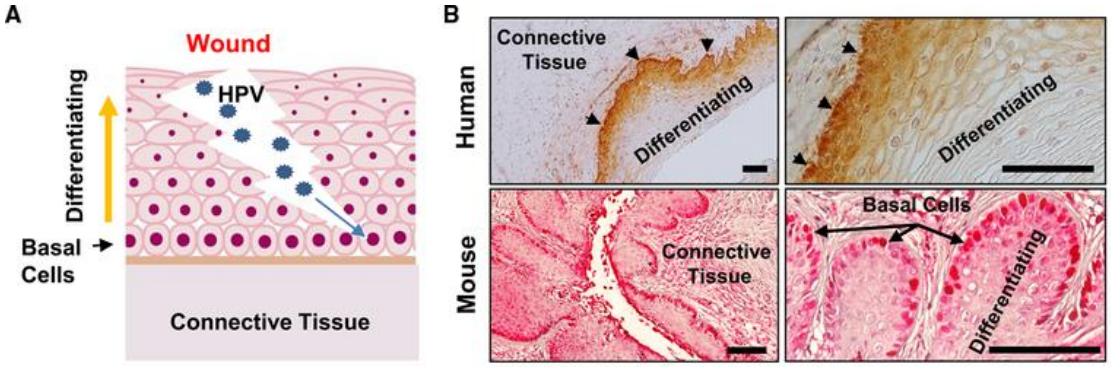

Figure. 6 (A) Natural process of human cervical HPV infection. (B) It shows the representative image of YAP1 (red) expression in normal cervical tissues of mice and human. YAP1 is found mostly in the cervical squamous epidermal cell nucleus.

6. Conclusion

In general, it is still a long way to go to study the Hippo-YAP signaling pathway. The conduction mechanism of YAP in the signaling pathway is expounded through various literatures on gynecological tumor experiments. Clinical trials and database data collection show that YAP is not only a tumor suppressor gene, but also an oncogene. In the future, YAP can be used as a therapeutic target to treat tumors and observe the distribution of YAP to predict the survival of these gynecological tumors. However, the existing clinical trials are still too few and need to be confirmed in a large number of patient populations. In addition, the drug resistance of YAP signal in tumor therapy (chemotherapy, immunotherapy, targeted therapy) is also studied, so as to achieve better effects in future tumor therapy. However, a large number of clinical experiments are still needed to prove that YAP inhibitor can be suitable for therapeutic methods with current technology and environment.

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