The Role of Cancer‐Associated Fibroblasts (Cafs) In Tumor Development

Feilu Qin [1, *], Keying Lan [2], Yue Sun [3]

[1] Sichuan Agricultural University, Yaan, China

[2] High School Attached to Northeast Normal University, Changchun, China

[3] Ocean University of China, Qingdao, China

* Corresponding Author Email: qinfeilu@stu.sicau.edu.cn

Abstract. Today, cancer is still the key threaten to life, and CAFs are one of the mostly studied risk factor. This article reviews the mechanism of respiration of CAFS cells in cancer like Reverse Warburg Effect of CAFs, as well as the mechanism of TGF-β/Smad, the related diseases and corresponding drugs by targeting CAFs production. In tumor microenvironment(TME), CAFs are closely associated with the development, transfer and aggression of tumor cells. 'Reverse Warburg Effect is one of the ways for CAFs to achieve metabolic cross-talk with tumor cells. Through oxidative glycolysis, CAFs can produce plenty of energy-rich substrate, like pyruvate, fat acid and lactate, those substrates are transported to mitochondria in tumor cells in order to produce many ATP by OXPHOS. Besides, CAFs transfer lactate in hypoxia by MCT4 and then MCT1 on tumor cells surface, which can support themselves. In addition, the TGF-β/Smad, the main pathway promoting the development of CAFs and tumor cells, is also dissected. By conducting a thorough analysis on the mechanism of TGF-β based malignant cells and CAFs production, the relevant drugs are introduced. Those findings can not only enhance our understanding of the complexity of tumor metabolism, but it also provides a theoretical basis for the development of new therapeutic strategies and potential targets.

Keywords: CAFs; Warburg Effect; TGF-β/Smad; Medicine.

1. Introduction

In recent years, the issue related to tumor is gradually increasing, which is originated from various factors, among these, people's unhealthy lifestyle is closely related to. With the development of tumor, it not only affects people's study and work, but also makes people's life more difficult due to the high price which is needed to keep health. In this regard, we decided to explore the mechanism of tumorigenesis. Through searching materials and reviewing a large number of literatures, we find that cancer associated fibroblasts is one of the reasons for promoting tumor generation.

First of foremost, what is a tumor cell? A tumor cell is an aberrant cell in a local tissue that loses its normal regulation of growth at the gene level under the control of various carcinogenic factors in the body, resulting in its clonal abnormal proliferation. Moreover, what is cancer associated fibroblasts that promote tumor growth? Cancer associated fibroblasts, referred to as CAFs, are one of the important components of tumor environment, which can promote tumor growth, invasion and metastasis and angiogenesis [3]. The basic feature of tumor cells: Contact Inhibition, Gene mutation and other clues cause unlimited proliferation; having rather aggressive and metastatic action; abnormal metabolism (Warburg Effect); immune escape jointly facilitated by expression of immunity inhibition factors, tumor microenvironment's adjustment and others; building of blood vessel led by requirements of metabolism, anoxic microenvironment and Metalloproteinase activation; Anchorage-Independent Growth.

According to statistics, the top 5 cancers in China are Lung cancer, Colorectal cancer/Esophageal cancer, Thyroid cancer, Liver cancer, Stomach cancer. These five cancers account for 67.5% of total cancer deaths. There will be approximately 4.827 million new cancer cases in China in 2022, with approximately 2.5339 million cases among men and 2.2908 million cases among women. According to statistics, the incidence is much lower between 0-34 age, with a significant increase in 35-39 ages

and reaching a peak in 80-84 age group. From the current trend forecasts, the number of new cancer cases and deaths worldwide is expected to increase by 49% and 62% respectively by 2040.As far as China is concerned, new cases are expected to reach 6.85 million and 5.07 million deaths, which emphasizes the future rise in cancer burden and changes in cancer spectrum [4]. As mentioned before, CAFs is one of the key risk factors of tumor generation. Under native state, the fibroblast is responsible for extracellular matrix production, which is important in maintaining the structural integrity of tissues, while in the tumor microenvironment (TME), tumor cells can secrete some transforming growth factors (TGF-β), fibroblast cytokines (FGF), interleukins (IL), etc., which promote the transformation of fibroblasts into tumor-associated fibroblasts (CAFs) [1]. t is known that the diversity of CAFs comes from different cell origins, the original fibroblasts and quiescent stellate cells can be activated, bone marrow-derived mesenchymal stem cells can be used as precursors of CAFs to differentiate into a large number of CAFs, epithelial cells can be transformed into CAFs through the epithelial-mesenchymal transition (EMT) process, and other cells such as pericytes, smooth muscle cells and adipocytes can also be transformed into CAFs through differentiation[2]. Among the different pathways resulting in EMT transmission, the one with TGF-β involved in will be discussed clearly. By taking several malignant tumors with highest incidence in China as an example, their molecular mechanisms of CAFs formation and adverse impacts will be deciphered thoroughly.

2. Some mechanisms of CAFs

2.1. Reverse Warburg Effect of CAFs

In Tumor microenviroment,as the primary tumor fiber stromal cells, CAFs are easily metabolic reprogrammed, which play an important role in tumor's growth, development and aggressive transfer. Therefore, it is important to study on the mutual influence between tumor cells and CAFs and the mechanism of metabolic reprogramming, which may contribute to develop new cure method and potential targets. Otto Warburg firstly put forward the concept of "Warburg Effect". Even in an aerobic condition, bad tumor prefers to use glycolysis to produce adenosine triphosphate (ATP) replacing OXPHOS, which is called "aerobic glycolysis". But actually, many cases are different with Warburg effect, some bad tumor cells tend to OXPHOS. Besides, plenty of researches have shown that OXPHOS and aerobic glycolysis may not be mutually exclusive. By the way, hypoxia and normoxia affect their contribution to ATP production in a great extent. We have mentioned that CAFs remarkably affects the tumor cells in growth, transfer, metabolism and development [14]. So, it's necessary to propose a model, "Reverse Warburg Effect" to take a new look at tumor's metabolism. Cancers cells secrete H2O2 into TME to heighten ROS, which can induce oxidative reaction in neighbouring cells. CAFs belong to stroamal cells, running aerobic glycolysis and make pyruvate, ketone, fatty acids and lactic acids, which are provided to mitochondrial in tumor cell return, and undergoing OXPHOS to get sufficient ATP. Among them, lactic acid is an important material [15,16]. Under hypoxia, lactic acid passes the lactic acid transporter monocarboxylic acid transporter (MCT)4 in CAFs, absorbed by MCT1 on tumor cells surface, which is distributed to mitochondria for OXPHOS, such that cancer cells can support themselves [17,18]. According to this model we can link the microenvironment of stromal cells with cancer cells. "Warburg effect" will continue under aerobic conditon. Pyruvate is turned into lactic acid with lactate dehydrogenase (LDH). Meanwhile, Extra lactic acid enters CAFs and distributed to mitochondria for OXPHOS, which can take lactate away from TME [19]. When TME is hypoxic, CAFs will experience the metabolic reprogramming of glycolysis, tumor cells get the material(lactate) and begin OXPHOS, which can promote their proliferation (as shown in figure 1) [20].

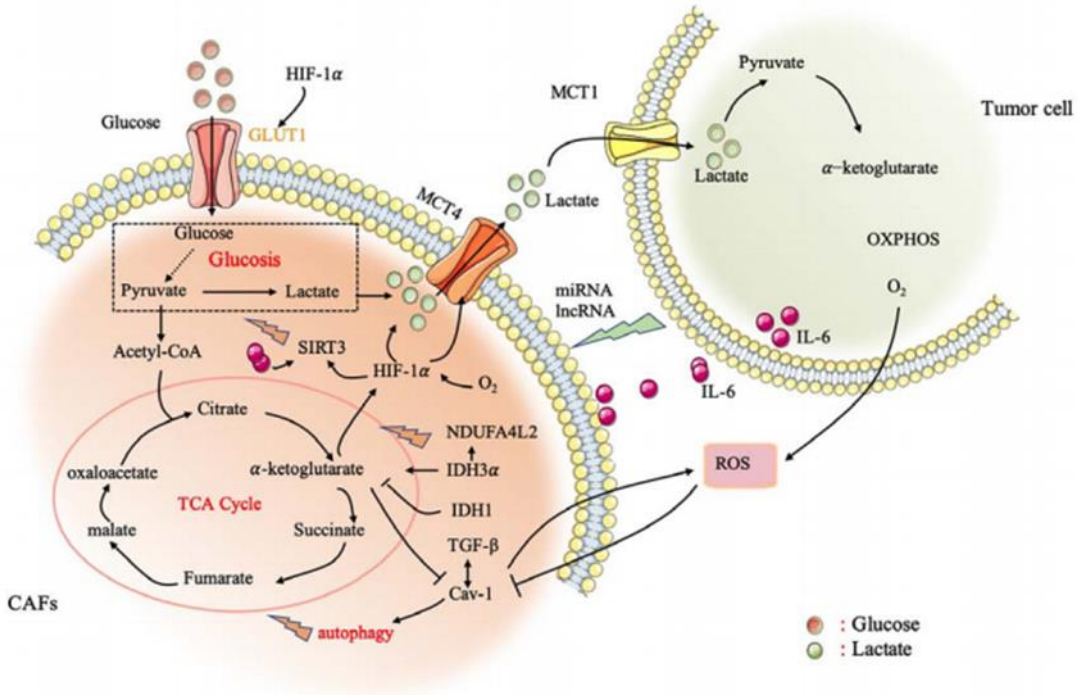

Fig. 1 The metabolism crosswalk between CAFs and tumor cells [29].

CAFs produce numerous energy-rich material (pyruvate,fatty acid and lactate).those materials will produce plenty of ATP through OXPHOS in tumor cell mitochondria. The lactate derived from CAFs transfers through MCT4,then cancer cells get those material by absorption of MCT1.High level of ROS secreted from cancer cells in tumor microenvironment will degrade Cav-1 protein,signaling of TGF-β and NF-kB are motivated,amount of IDH1decrease,the activity of PHD is lower,the hydroxylation of HIF-1α is suppressed,then aerobic glycolysis is triggered,affecting mitochondria oxidative phosphorylation.In addition, IncRNAs and miRNAs ,as biological molecules that are newly found also play an important role in mutual affection between CAFs and cancer cells, which affect reprogramming of metabolism.

In the model of ‘autophagic tumor stromal model of cancer metabolism’, the high concentration of ROS in TME induce the oxidative reaction in CAFs, starting the secretion of autophagosomes mixed with lysosomes, which can degrade cavolin-1(Cva-1) and mitochondria in turn. Next, the absence of Cav-1 accelerates ROS, motivating oxidative stress response in CAFs with a positive feedback mechanism [21].

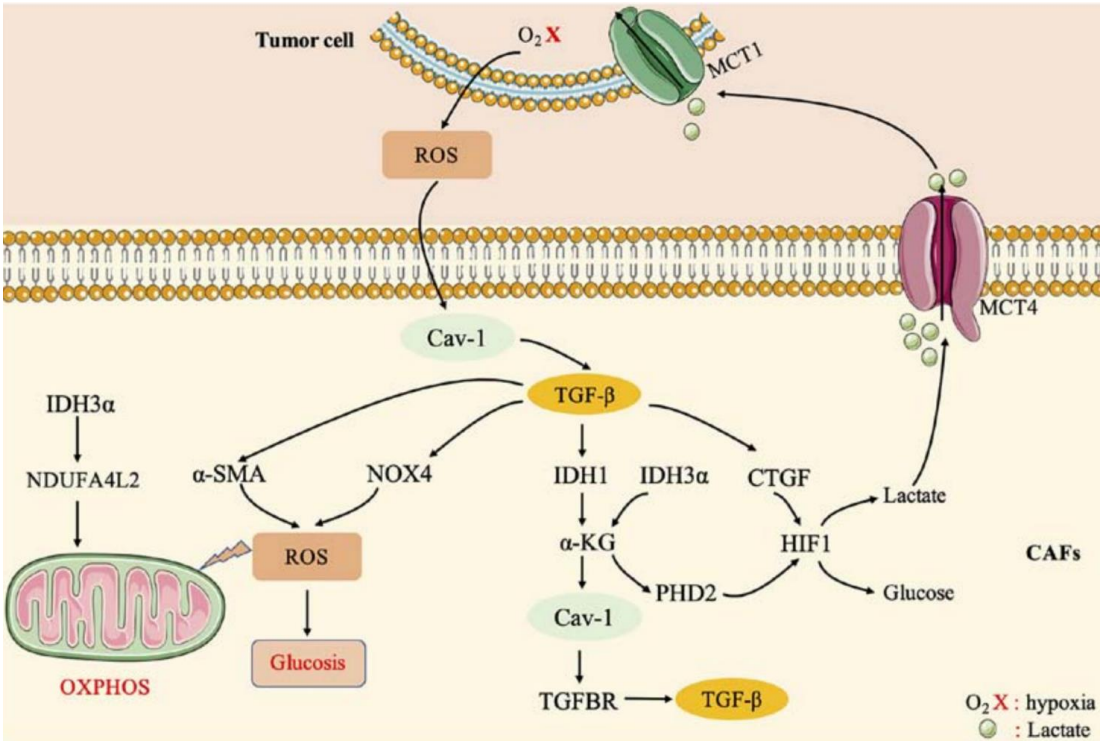

Fig. 2 The TGF-β signaling regulated by Cav-1 [29].

Some studies have shown that the trigger of TGF-β signaling, deactivation of tumor suppressor gene and oncogenes are connected with the knockdown of Cav-1. As known that Cav-1 is a negative regulatory factor derived from NADPH oxidase (NOX), Cav-1 inhibit the activation of NOX2 and NOX5 by directly combining with these enzymes to reduce production of ROS. Besides, the knockdown of Cav-1 inhibits expression of prolyl hydroxylase domain protein (PHD), who mediates the activation of NF-kB signaling that promote gene expression and protein synthesis of NOX2 and NOX4.

HIF-1α and NF-kB are particular necessary. The reduction of PHD expression become a trigger of activation of HIF-1α and NF-kB signaling [22]. Cav-1 gene down-regulated induce the expression of PKM2, which can directly influence the HIF-1α, promoting the combination with HRE (hypoxia response element) by enhancing the accumulation of p300 then achieve the transactivition of HIF-1α target gene (VEGF-promoting blood vessel building, GLUT1-glucose transporter protein, LDHA-lactic dehydrogenase).

Moreover, JMJD5 interacts directly with PKM2, blocking PKM2 tetramerization and pyruvate kinase activity makes PKM2 moving to nucleus and promote HIF-1α-mediated target gene activity and reprogramming the metabolism progress in tumor cells. Many statistics have shown that signaling motivated by CAFs is up to NF-kB in certain extent, and it has central role in mediating early period of tumor growth.

We need to consider deeply about the mechanism of signaling path of CAFs and tumor cells. Cancer cells secrete high level of ROS can trigger the oxidative stress response, producing the autophagosomes mixed with lysosomes, destroying mitochondria then degrading Cav-1[23]. One of the features of CAFs in tumor microenvironment is the degradation and gene mutation of Cav-1. The knockdown of Cav-1 leads a higher level of ROS, which can build a positive feedback way to induce oxidative stress response. Moreover, the knockdown of Cav-1 may be related to inducement of TGF-β signaling and it can cause the decline of IDH1, permitting the accumulation of α-Ketoglutaric acid, causing the death of Cav-1.

HIF-1α and NF-kB, the activity of signaling generates the transcription of glycolysis gene. Under aerobic condition, the subunit of HIF-1αis dehydroxylated, and being degrading by E3 connecting enzyme and HipprlLindau(VHL) protein. The high level of ROS in hypoxia can suppress the activity of PHD, declining the rate of dehydroxylation of HIF-1α. HIF-1αinduces hypoxic environment and

promote the transcription of factor of building blood vessel, mediating autophagosome, autophagosome of mitochondria and aerobic glycolysis, which play a key role in affecting reprogramming of metabolism. Besides, HIF can also regulate some genes that interact with glucose metabolism, such as MCT4 and programming the genes of glucose transporter protein (GLUT1, GLUT3) to rise the uptake of glucose, which influence transportation of lactate. In addition, Isocitrate Dehydrogenase3(IDH3), in CAFs cells mitochondria, as an important rate-limiting enzyme in Citric Acid Cycle and induce the production of α-KG. In order to decline level of IDH3 we can decrease the rate of Fumaric acid and Succinic acid, which it's useful to suppress the activity of PHD2. By the way, the level of dehydroxylation of HIF-1αis stimulated also the stability is enhanced, which can restrain the inducement of mediating the transcription and reprogramming. A negative regulatory factor (NDUFAL2) may restrain OXPHOS, those clues indicate that IDH3 can be a important trigger of reprogramming metabolism of CAFs.

2.2. Signal transduction of the TGF-β1/Smad pathway

Tumor cells can secrete TGF-β paracrinely, acting on fibroblasts in the TME, thereby transforming into CAFs [4]. Three types of TGF-β have been recognized in mammals: TGF-β1, TGF-β2, and TGF-β3, and the similarity of these three factors is high [5]. TGF-β receptor (TGFβ-R) is divided into two types, TGFβ-R type I and type II. It is very essential in EMT and fibrosis [6]. TGF-β binds to TβR-II and undergoes phosphorylation modification to activate TGFβ-RI. Phosphorylated Smad2, Smad3 form a trimer complex with universal Smad4. This complex enters the nucleus and cooperates with corepressors, coactivators, and other transcription factors to control the expression of particular genes [5]. SMAD3 binds to the promoter region of collagen to promote renal fibrosis. Furthermore, the synthesis of tissue inhibitors of metalloproteinase-1 (TIMP-1) and the inhibition of matrix metalloproteinases-1(MMP-1) activity in fibroblasts were found to limit the degradation of extracellular matrix (ECM). Smad4 is a universal Smad in the TGF-β and osteogenesis signaling pathways, and Smad4 deletion does not affect the phosphorylation of Smad2 and Smad3. The inhibitory regulator Smad7 has the effect of competitively binding TGFβ-RI [7]. to Smad2 and Smad3, blocking the binding of Smad2 and Smad3 to receptors, thereby blocking TGF-β1 signaling [7].

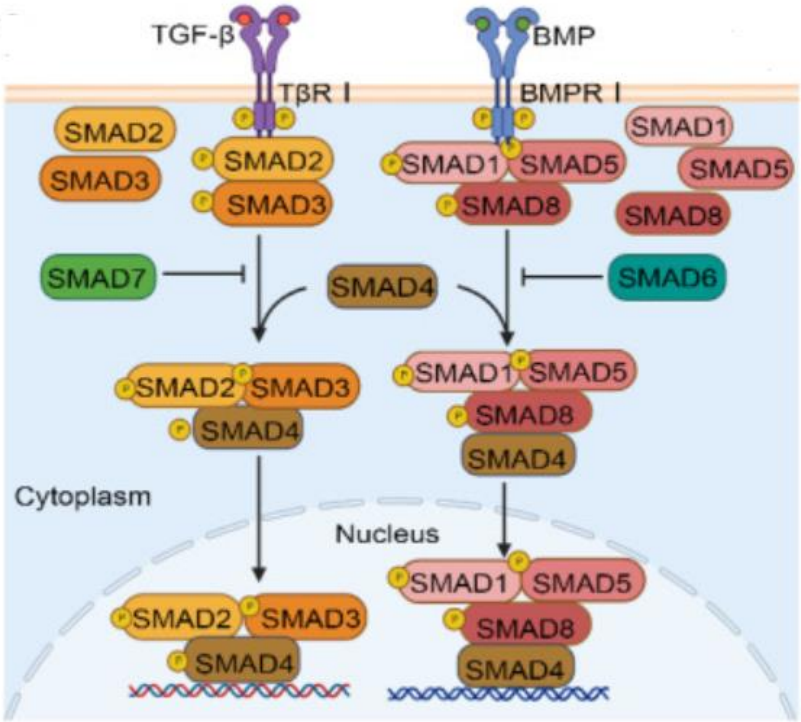

Fig. 3 When the TGF-β ligand binds to TβRI, the TGF-β signaling cascade is initiated [30].

2.3. Drug Mechanism

2.3.1 Functions of TGF-β in tumorigenesis

TGF-β, as a growth factor, functions differently in the context of the development of tumors may differ depending on the specific stage of tumor growth. On the one hand, it can induce precancerous cell apoptosis, inhibiting the proliferation of cancer cells, while, when the TGF‐β signaling pathway dysfunctions, it will cause a variety of diseases, such as tissue and organ fibrosis, cardiovascular disease, cancer, bone and articular cartilage inflammation, autoimmune diseases, neuropathy, etc. On the other hand, in advanced stages of tumor cells have the capability to either suppress the TGF-β pathway or develop resistance to TGF-β. This enables TGF-β to promote the development of tumor microenvironment transformations (TMT), facilitating tumor invasion and metastasis.

2.3.2 Drugs target to CAFs

To find related drugs that specifically target CAFs, and introduce the mechanism of this drug and its effect on the corresponding tumor treatment. There are currently related drugs that specifically target CAFs production: (1) antisense oligonucleotides directly inhibit TGF-β synthesis; (2) Instead of using monoclonal antibodies or soluble TGF-β TRAP, researchers are investigating the inhibition of TGF-β and its receptor with alternative methods; (3) Kinase inhibitors or aptamers that interfere with downstream TGF-β signaling protein function inhibit the TGF-β signaling pathway

A) Antisense oligonucleotides (ASOs)

It's made up of the DNA of the genes in our body, and in that DNA there's a special code that allows cells to function, and we can use that information to create proteins that maintain cell function, ASOs and it can interfere with DNA and proteins. Trabederson AP12009 is a synthetic 18-thiophosphate ASOs. We discuss the development of trabedersen (AP 12009), an antisense oligodeoxynucleotide designed to specifically cement the production of TGF-β2. Through in vitro and in vivo experiments, the mechanism of action, efficacy and tolerance of trabedersen have been validated, leading to its progression in clinical trials. A pivotal phase III study is evaluating trabedersen's intratumor treatment for high-grade glioma, while a phase I/II dose-increase study is investigating intravenous trabedersen for patients with advanced pancreatic carcinoma, metastatic melanoma, and metastatic colorectal carcinoma are being studied in clinical trials [25].

B) Anti-tgf-β tumor vaccine

Belagenpanatucel-L functions by introducing pancreatic cancer cells into the body to stimulate the patient's immune system to identify these cells as alien and trigger an immune response against them. GM-CSF promotes the stimulation of immune cells that display antigens, like dendritic cells, such as dendritic cells, enabling T cells to more effectively identify and destroy cancer cells. This approach may enhance the patient’s immune resistance to pancreatic cancer and inhibit tumor growth and metastasis.

C) TGF-β antibody

Fresolimumab binds to EGFR and blocks its interaction with natural ligands, thereby inhibiting the cell proliferation and survival signals triggered by EGFR activation. In NSCLC, where the abnormal activation of the EGFR pathway is commonly linked to tumor progression and spread, Fresolimumab has demonstrated effectiveness in clinical studies by prolonging the survival of specific patient groups.

Bintrafusp alfa: Bintrafusp alfa, a new fusion protein, is being studied for its safety and efficacy in inhibiting the TGF-β pathway by combining the extracellular domain of TGF-β receptor II with a human immunoglobulin G1 antibody [26].

D) Small molecule inhibitor of TGF-βR kinase activity

Vactosertib is an inhibitor, Vactosertib is a safe immunotherapy that modulates the t cell immunophenotype, making it healthy for rejuvenated t cells [27].

Galunisertib:Galunisertib, an oral small molecule inhibitor of TGF-beta receptor type 1 (TGF-beta-RI), is used to disable TGF-beta classical pathway activation and has shown improvement in OS in patients with unresectable pancreatic cancer [28].

3. Discussion

In this study, we identified the mechanism of reverse Warburg effect of CAFs, signal transduction of the TGF-β1/Smad pathway and functions of TGF-β in tumorigenesis. Consistent with previous study, we find TGF-β binds to TβR-II and undergoes phosphorylation modification to activate TGFβ-RI, phosphorylated Smad2, Smad3 form a trimer complex with universal Smad4. Additionally, In the experiment, these drugs also showed problems related to treatment, and I think the most important thing is that the treatment did not improve survival and overall survival, and there was also uncontrolled CRS which could not be caused by the treatment.

We deeply discussed the mutual metabolic effects between the tumor microenvironment and CAFs cells, especially the affection of 'Reverse Warburg effect' in tumor metabolism. We summarize many factors in CAFs that potentially mediate or induce the tumor development, transfer and reprogramming of metabolism, like the regulation of HIF-1α and NF-kB, secreting ROS to promote oxidative stress response. Although some specific mechanism in those affection, we hope that it can contribute to cancer treatment somehow.

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