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Tumor Microenvironment Can Promote Cancer Cell Invasion and Foster Immune Suppression

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Abstract. Immunotherapy against tumors is an emerging field and it is gaining more attention. However, the efficiency of immunotherapy can be hindered by the complexity of the tumor microenvironment. In this paper, the mechanisms by which tumor microenvironment affects immune cells cytotoxicity and how it might suggest new treatment regimes were discussed. In addition, several new in vitro experiments were listed that point to new directions of supplementary treatments to enhance immunotherapy.

Keywords: Tumor microenvironment, immune cells, tumor cells, stiffness in tumor microenvironment, cell tension force, extracellular matrix.

1. Introduction

For many years, cancer has been a critical health problem for premature death worldwide. In 2018 alone, it accounted for about 9.6 million deaths across the world[1]. With the advancement of clinical detection technologies and the extent of environmental pollution, cancer is becoming more prevalent. Cancer treatments are oftentimes very expensive, despite the disputable efficiency. According to the data from the National Cancer Institute (NCI), there were 19.3 million new cancer cases and nearly 10 million cancer-related deaths worldwide in 2020 and the average cost to treat cancer is 105,500 - 150,000 dollars per patient[2]. Furthermore, for some patients, even though they are cured from the cancer, they may still have sequelae and other long-term suffering because of the cancer treatment, which will bother them for the rest of their life[3]. Immunotherapy presented as a promising method for cancer treatment and preventing cancer remission[4].

In addition to traditional methods of surgery, radiotherapy, and chemotherapy, immunotherapy arises as a more targeted therapy. Immunotherapy, in a simple term, is using your own immune system to target and destroy cancer cells. Its discovery, though, can be dated back to ancient Egypt and the 19th century, where there have been several reports of tumors disappearing after infecting the patients with bacteria that caused concomitant high fever[4]. Mounting evidence has suggested that tumors can be clinically evident only when the responsive immunological surveillance has failed[5]. Therefore, reactivating and boosting immunotherapy has been the key and new battle ground in cancer treatment.

Two major breakthroughs in immunotherapy are immune checkpoint inhibitors and adoptive immunotherapy. Immune checkpoint proteins, like PD-1L, exist on cancer cell surfaces and immune cells in tumor microenvironment, and they can bind to suppressive surface proteins such as PD-1 on T-cells and block the T-cell killing activity[6]. By using inhibitors against these immune checkpoint proteins, T cells are able to recognize the cancer cells via major histocompatibility complex class-1 (MHC-1) molecules presented by antigen-presenting cells (APCs) and formation of immunological synapses and re-engage in cytolytic activities[7]. In addition, the breakout of the covid-19 caused great improvement in mRNA vaccine technology, and it can also be used as treatments for cancer[8]. Another type of immunotherapy that has gained great success in the treatment of beta cell malignancies like leukemia and lymphoma is the CAR-T cell therapy, which involves extracting the patients' own T cells, modifying T cells with genetically engineered receptors (chimeric antigen receptors (CAR)) which targets specific antigen on cancer cells and then injecting them back to patients[9].

Even with the advancement of immunotherapy, it still has limitations and does not support fully effective cancer treatment. For instance, immune infiltration by immune effector cells is sometimes hindered in some solid tumor cases. Researchers found out that the extracellular matrix can prevent the immune infiltration from happening physically and cancer cells can prevent cytotoxic T lymphocytes from maturing by secreting different kinds of signals or limiting the available nutrients. In addition, the immune cells which infiltrate successfully do not work effectively due to immune evasion caused by cancer cells and tumor microenvironment[10].

Tumor microenvironment (TME) is composed of malignant cancer cells, stroma and fibroblasts, extracellular molecules secreted by the cells, as well as immune cells. TME can cause immune suppression in various ways, including increasing the expression of certain cell surface molecules by T cells such as PD-1 and CTLA-4, which can suppress the function of T cells. TME can also increase the production of cytokines, which hinders the infiltration of T cells and T cell cytotoxicity. TME can also alter the chemical microenvironment by producing chemicals such as adenosine and lactate, which inhibits T cell function and proliferation. It is worth noticing that the PD-1 signaling pathway mostly works in TME, where its ligands are commonly overexpressed by tumor cells[11]. Therefore, TME, as an emerging topic in cancer research, plays important roles in modifying immune infiltration, causing immune suppression and immune evasion. More and more research supports the idea that TME plays a critical and indispensable role when developing proper treatments of tumors. The study of TME has developed in the past few years and is gaining more and more attention. In this paper, we are going to focus on the TME's influence on both immune cells and tumor cells and its roles in immune infiltration, immunosuppression and immune evasion. In this paper, we hypothesized that TME promotes cancer invasion, immune evasion and prevent immune infiltration from happening and the mechanisms of that can be studied to improve the efficacy of immunotherapy.

2. Results

2.1. TME fibrosis can promote cancer cell invasion and migration

Research in various types of tumors showed TME fibrosis is a hallmark of tumor progression and metastasis. In fact, many studies have shown that epithelial-mesenchymal transition of TME cells facilitate the invasion of cancer cells[12]. The findings are summarized in Table 1. The main culprit of TME fibrosis is cancer associated fibroblasts. Oftentimes the extent of TME fibrosis can be used as a predictor for prognosis and used in management of cancer treatment.

Pancreatic Ductal Adenocarcinoma (PDAC), among various types of cancers, is an aggressive pancreatic cancer which is characterized by the presence of extensive desmoplasia, which is thought to be responsible for the poor response of patients to systemic therapies. However, the PDAC TME is a complicated system, as depletion of myofibroblasts will lead to tumor invasion and decreased survival[20]. Instead, there are extensive studies that have shown that remodeling of TME can facilitate cancer treatment. PDAC's TME fibrosis suppresses the immune response by recruiting the immune suppressive immune cells, such as T-regulatory cells (Tregs) and tumor associated macrophages (TAMs), and block the infiltration of tumor suppressive immune cells such as CD4+ and CD8+ T cells (Figure 1)[21]. In addition, research in Hernandez lab has shown that pancreatic stellate cells in the TME can remodel the extracellular matrix (ECM) and promote cancer cell invasion (Figure 2)[22].

2.2. TME affects immune cell infiltration and lowers immunotherapy efficiency

For the tumors which the immune infiltration cannot take place, the scientists named them as the "cold tumors." There are several ways which tumors use to stop the immune infiltration from happening, such as removing strong immunogenic neoantigens, inhibiting dendritic cells from maturation with tumor derived-factors (such as IL-10, macrophage colony-stimulating factor (MCSF), prostaglandin, TGF-b and indoleamine 2,3-dioxygenase (IDO)). Tumors can also suppress T cell activities by reducing the expression of co-stimulatory factors and MHC to limit the co-stimulation

required for T cells, or inhibit T cell migration by reducing the expression of CXCR3 ligands such as CXCL9, CXCL10, and CXCL11[10]. In addition, tumors transform the nearby blood vessels by secreting neoplastic factors such as VEGF, reducing the expression of adherent factors in endothelial cells. At last, the immunosuppressive immune cells and cancer-associated fibroblast (CAF) produce extracellular matrix (ECM) to suppress T cells physically and secret chemokines such as CXCL12 to inhibit the migration of T cells to tumors[23].

2.3. TME can be reprogrammed and reverted back to a state that does not favor tumor progression

TME is crucial to both immune cell function and cancer cell activities in the case of PDAC. As a result, it is possible to target TME as the new treatment direction. As shown in Figure 3 and 4, studies by Lachowski et al. showed that pancreatic stellate cells (PSCs), which are the key TME cells around PDAC tumor, can sense the pressure around them and be conditioned and revert back to quiescent state[24]. The research by Lachowski's team suggested that new research aimed to understand how matrix stiffness and mechanotransduction could break the positive durataxis feedback loop and lead to better immune cell infiltration. According to various research studies, G protein-coupled estrogen receptor (GPER) and retinoic acid receptor b (RAR-b) play an important role in inhibiting TME stiffness generation in both cancer cells and pancreatic stellate cells (PSCs)[22]. Figure 5 showed how Cortes et al. used tamoxifen as a treatment that can target and modulate the mechanotransduction of PSCs. In the aspect of PSCs, it modifies the PDAC TME in three ways. First, the stiff matrix in TME induces PSC activation and causes a positive feedback loop that perpetuates PSC activation and stiffness[25]. Secondly, by adding ATRA, one can reprogramme the cells in the TME back to quiescent state, thus reverting the positive feedback loop[26]. Last but not least, quiescent PSCs help the activation and increase effector T cell killing[27].

2.4. Increasing cancer cells' surface tension and rigidity can facilitate CTL killing

Basu et al. discovered that cytotoxic T cells might use mechanical force to increase cytotoxicity and perforin pore formation speed (Figure 6)[28]. Furthermore, research from Ekrem Emrah Er's lab showed that high expression of myocardial-related transcription factors (MRTFs) make cancer cells more vulnerable to cytotoxic T lymphocytes (CTLs) and natural killer cells, possibly through increasing cancer cells rigidity and making them more vulnerable to CTLs[29]. Recent research led by Kewen Lei showed that cancer-cell stiffening by cholesterol depletion can augment T cell cytotoxicity and enhance the efficacy of adoptive T-cell therapy against solid tumors[30].

3. Increasing cancer cells' surface tension and rigidity can facilitate CTL killing:

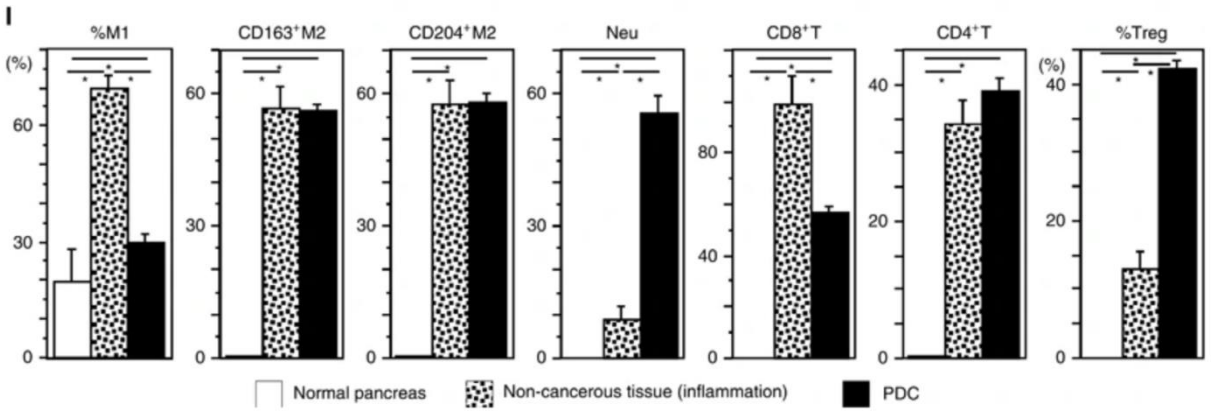

Fig. 1 Comparison of percentage of infiltrating immune cells revealed by immunohistochemical staining in normal pancreas, chronic pancreatitis (non-cancerous pancreas), and pancreatic ductal carcinoma (PDC)[21].

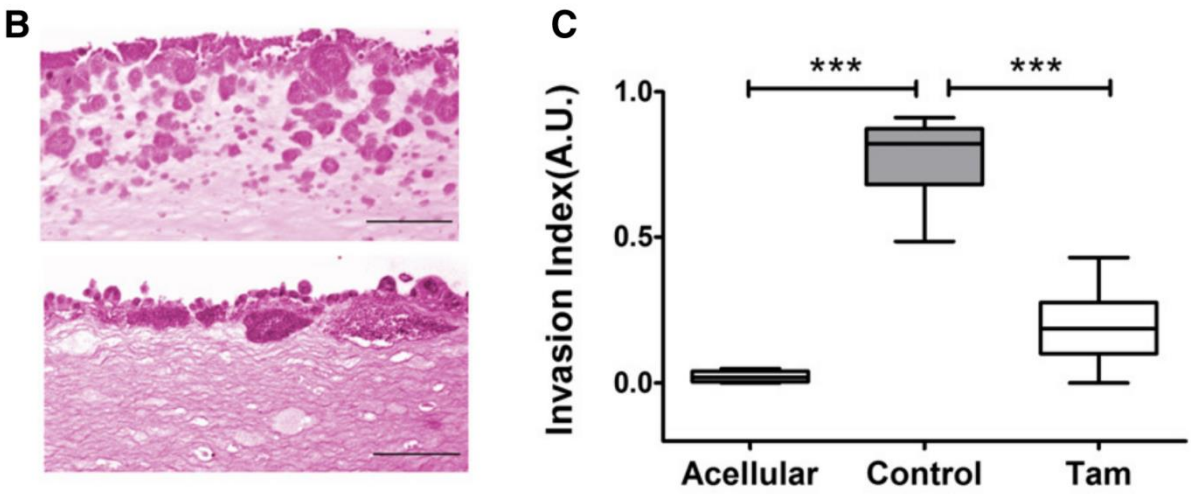

Fig. 2 Tamoxifen can suppress ECM remodeling. The left depicted representative images of H&E staining showing cancer cell invasion in remodeled matrices by PSC (top) and tamoxifen treated PSC (bottom). The right figure showed quantified invasion index comparison between control (PSC-remodeled matrices) and Tam (tamoxifen treated PSC)[22].

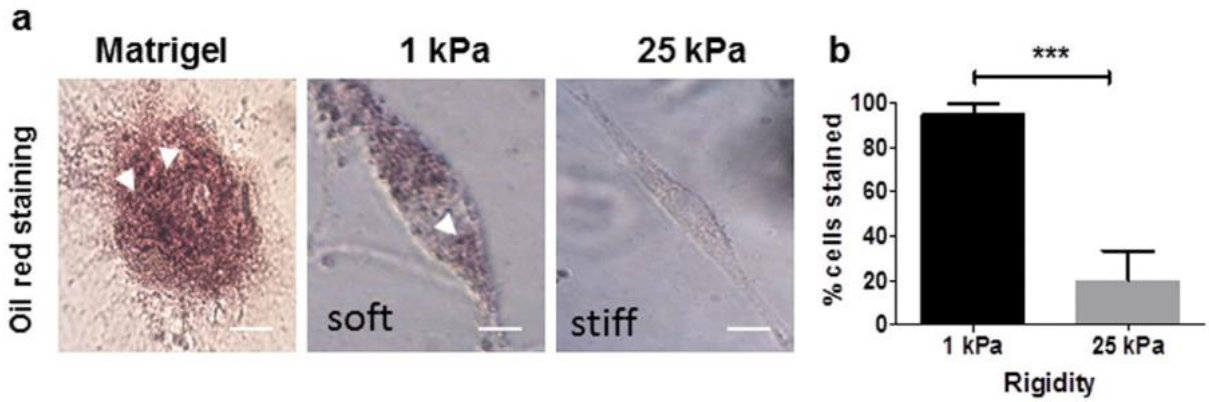

Fig. 3 PSCs can sense the pressure around and PSCs placed onto the soft metrices retained the ability to store lipid droplets.

To study the relationship between the environment and the activation of PSCs, the team placed Matrigel-induced quiescent PSCs onto PAA hydrogels resembling soft (1kPa) and stiff (25kPa) tissues. The team also used Oil Red staining to identify the presence of any cytoplasmic lipid droplets, which is the characteristic of PSC quiescence. They observed that the PSCs placed onto the soft metrices retained the ability to store lipid droplets (Figure 3a). Quantification of seeded cell populations presented a statistically significant difference in Oil Red staining levels (95% stained on soft hydrogels and 20% stained on stiff hydrogels) (Figure 3b). Based on the observation, the substrate stiffness can induce phenotype transition of PSCs to a fibroblast-like active state[24].

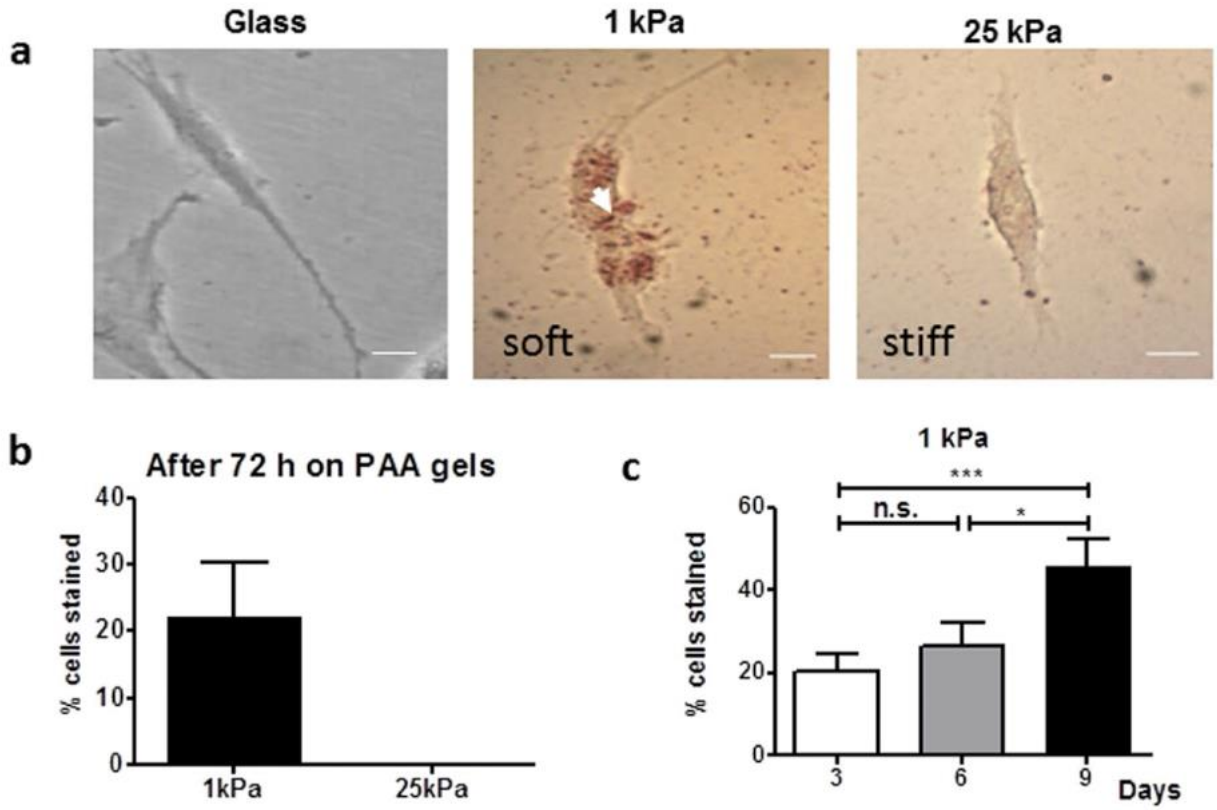

Fig. 4 PSCs grown on glass can be reverted back to quiescent state by plating on soft matrices.

These experiments indicate that the soft environment can induce quiescence in PSCs. The team transferred previously glass culture-activated PSCs and grew them on soft and stiff hydrogels for three days and observed the PSCs grown on soft matrices began to regain cytoplasmic lipid droplets (Figure 4a). Quantification of this experiment showed that 22% of the previously culture-activated PSCs reverted to a state of quiescence after 3 days of culture on soft hydrogels (Figure 4b, 4c)[24].

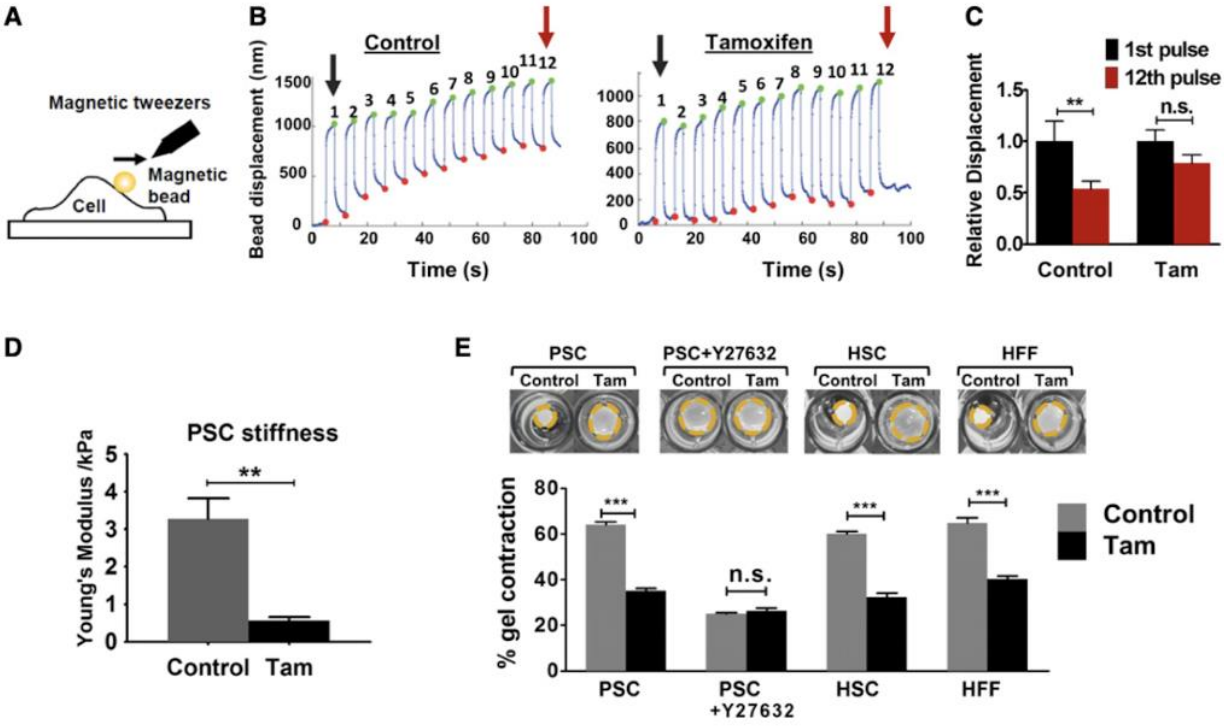

Fig. 5 The effect of tamoxifen on PSC's ability of mechanosensing and force generation.

The team performed experiments with PSCs treated with 5 um of tamoxifen or vehicle control for 10 days. They also use a magnetic tweezers device to apply a pulsatile force regimen on cells' integral receptors to test PSCs' ability to sense a mechanical external stimulus (Figure 5A). Cells with an intact mechanosensing ability normally detect force application and respond to it by rapidly remodeling and stiffening their cytoskeleton. The control PSCs showed a decrease in the oscillatory amplitude of the bead bound to the cell, the tamoxifen-treated PSCs displayed a significantly impaired mechanosensing (Figure 5B, 5C). Meanwhile, tamoxifen-treated PSCs were significantly softer compared to control PSCs, suggesting a decrease in overall cytoskeleton tension (Figure 5D). The ability of fibroblasts to contract collagen gels correlates with their ECM remodeling capacity. To find out how tamoxifen affects the ability of PSCs to apply forces, the team placed PSCs in 3D collagen I/Matrigel. The control PSCs significantly contracted the gels after 72 h (65% gel contraction) while treated PSCs showed a severe reduced contraction (35% gel contraction).

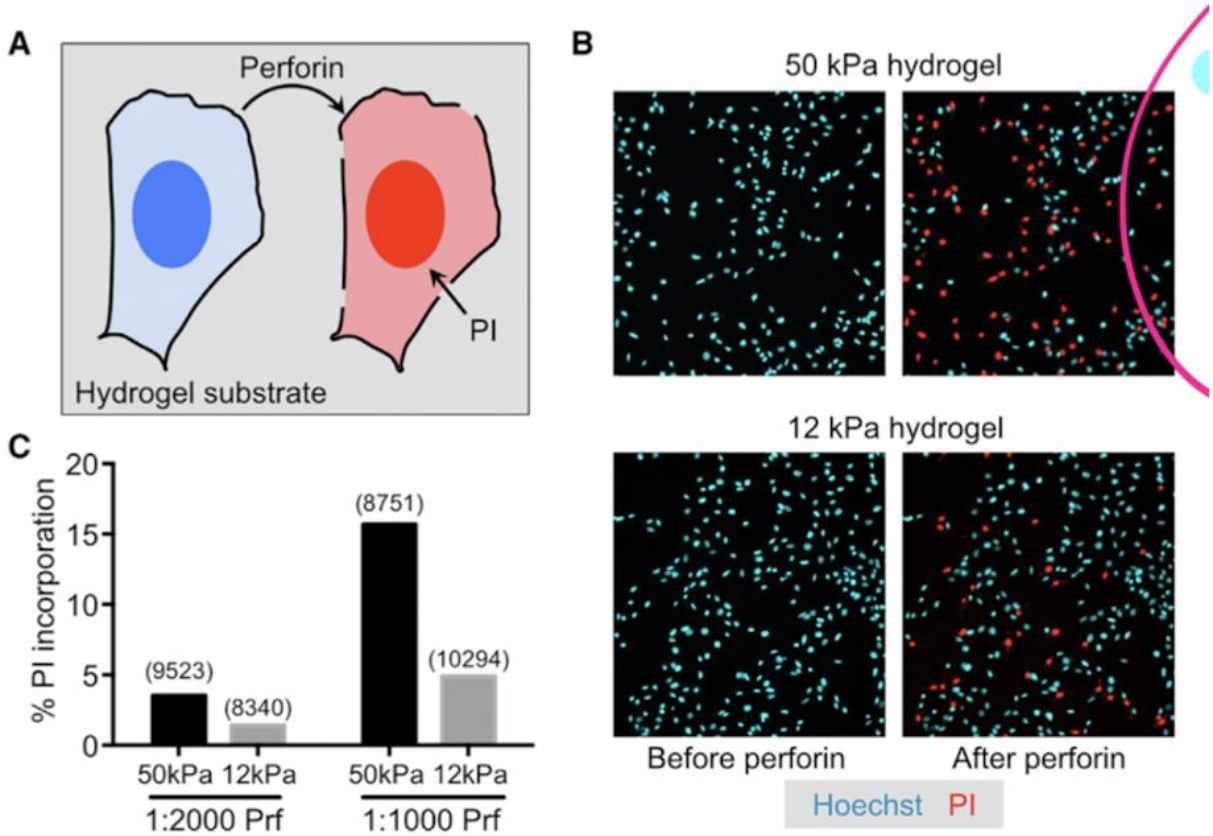

Fig. 6 Experiments to explore the relationship between cell tension and perforin function.

The team grew adherent cells on polyacrylamide hydrogels of varying stiffness and the stiffness of hydrogels reflects different cell tensions. The team tested the cells with purified perforin protein in the presence of propidium iodide (PI) which will render the target cell fluorescent when accessing the cytoplasm (Figure 6A). The team observed that cells on 50kPa substrates were more sensitive to perforin than those on 12kPa substrates. This imply that increased cell tension can potentiate perforin activity (Figure 6B, 6C).

Table 1. Summary of studies of TME's contributions in various types of cancer.

Type of tumor	Summary of findings	Reference

Breast cancer	Pathological assessment of TME has been incorporated into routine breast cancer diagnosis and prognosis analysis. In essence, fibrosis in TME is associated with shorter prognosis and worse outcomes.	Joshua Li, et al.[13]
Colorectal cancer	TME remodeling through cancer associated fibroblasts (CAFs) can promote tumor angiogenesis and migration.	Jun Li, et al.[14]
Ovarian cancer	Proteins expressed by CAFs enhanced the migration and invasion of ovarian cancer cells.	Yanfei Yang, et al.[15]
Pancreatic cancer	Desmoplasia or dense stroma formation promotes cancer cell proliferation, invasion and metastasis.	Takashi Murakami et al.[16]
Prostate cancer	Crosstalk between epithelial cells and stroma in TME of prostate cancer is very important for disease progression and tumor metastasis.	Hisham F. Bahmad, et al.[17]
Renal cell cancer	Renal cell carcinoma microenvironment can promote the growth and metastasis of the tumor cells, and it can undermine the immune response.	James W. Mier.[18]
Lung cancer	Hypoxia in TME of non-small-cell lung cancer can correlate with worse radiation responses.	Edward E. Graves, et al.[19]

4. Conclusion

In this paper, the importance of TME on cancer treatment, especially on PDAC, were discussed and several mechanisms were summarized. Based on these mechanisms, some potential new treatments were listed and discussed.

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