From Microbiology, Tumor and Cell Biology
Karolinska Institutet, Stockholm, Sweden

TARGETING STROMAL COMPONENTS IN THE TUMOR MICROENVIRONMENT FOR CANCER THERAPY

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Karolinska Institutet

Stockholm 2022

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Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2022
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ISBN 978-91-8016-899-1
Cover illustration: The topics in the thesis, including angiogenesis, cancer-associated adipocytes, cancer-associated fibroblasts, tumor-associated macrophages, and the circadian rhythm of cancer host (drew by Jieyu Wu with software Sketchbook).

Targeting stromal components in the tumor microenvironment for cancer therapy
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at Inghesalen, Widerströmska, Solna, 2023-01-13 at 14:00

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To my family

Thank you for the unconditional love and support.

献给我的家人，感谢你们对我的爱与支持。

POPULAR SCIENCE SUMMARY OF THE THESIS

Similar to the parasite plants grown in an appropriate host in a good environment, the cancer tissue grown in a certain microenvironment that could support their multiplying and spreading. This environment of cancer grown is called tumor microenvironment (TME). We continue to imagine the TME as the environment of parasite plants, there are many players in this scenario. For example, the vasculature structure transports nutrition and oxygen as the roots. The inactive immune system is like a lazy predator that let the parasite plants grow wildly. The cancer-associated fibroblasts promoted cancer remote invasion are acting like pollination by insects. They carry the cancer cells to other organs. Even the cancer is affected by the sun rise and set, since the circadian clock also controls cancer. Meanwhile, the components of TME can crosstalk with each other, as well as the cancer cells, to produce growth factors and cytokines, just like chemical fertilizers, to trigger cancer growth. Our results of this thesis provide approaches to target on the TME, which offer novel ideas for cancer therapy.

In **Paper I**, we investigated the reasons results in antiangiogenic drugs (AAD) resistance in the cancer adjacent to fat tissue. The AAD drugs induced vessel regression in cancer. Lacking vessels, the TME showed extremely low oxygen; we called it hypoxia. We found that hypoxia switched the cancer to uptake free fatty acid instead of glucose (FFA). The FFA provided energy support for cancer cells growth. Therefore, we used drugs to shut down the FFA transporter and FFA could not enter cancer cells. Then the AAD resistance effect reversed. Our data suggested a therapeutic idea to reverse the AAD resistance in the cancers.

In **Paper II**, we invented an approach to enlarge and activate the brown fat in adults. There are two types of fats in mammal bodies, the white fat and the brown fat. The brown fat has a high ratio mass in fetal, however, as we become adults, the brown fat mass become tiny. Studies found that the activation of brown fat could burn down the white fat, making body weight loss and decreasing blood sugar level. Our previous study found that brown fat could even compete for energy uptake with cancer. Therefore, making a larger brown fat with high activation could be a way to treat cancer. In this study, we blocked the brown fat stem cell gatekeeper protein PDGFRα in mice with antibody and drugs. We found that after PDGFRα blockage, the brown fat cells become smaller, but the whole tissue mass was mega because there were more stem cells turned into mature brown cells. The obese mice with this treatment had their brown fat became mega brown fat. Their glucose level, insulin tolerance and blood lipids level were markedly improved. Even the steatotic livers in these mice were reversed. The mega brown fat is a potential therapeutic approach in obesity, type II diabetes and cancer.

In **Paper III**, we revealed that high FGF-2 expression cancer is resistant to conventional anti-cancer vessel therapy. There are many proteins that trigger cancer vessel formation, for example VEGF, FGF-2 and PDGF. FGF-2 induced high PDGF protein that could recruit pericytes to cancer vessels. Pericytes are the cells normally located around the vessels to maintain vessel structure and function. However, over coverage of pericytes in the cancer vessels result in preventing drug effects. Therefore, high FGF-2 expression cancer has no effect on mono anti-VEGF or anti-PDGFR therapy. So far, there are no effective FGF-2 drugs. In this

study, we used a combination therapy with anti-VEGF and anti-PDGFRβ drugs in high FGF-2 cancer. It showed a remarkable effect, since anti-PDGFRβ suppressed pericytes recruitment and anti-VEGF could directly target on cancer vessels. Finally, the cancer mass shrank with cancer vessels regression.

In **Paper IV and V**, we found the crucial role of cancer-associated fibroblasts (CAFs) in cancer growth and migration. In paper IV, we investigated the pancreatic ductal adenocarcinoma (PDAC), which is so far the most difficult cancer to treat in the world. We found that in PDAC, cancer CAFs and pericytes produce an IL-33 protein. This protein binds to its receptors on tumor-associated macrophages (TAMs), then TAMs produce another protein CXCL3. The CXCL3 as a signal reversely talked with CAFs by binding to its receptors on CAFs. With this signal, CAFs aggressively multiplied. Meanwhile, the CAFs gain a new phenotype with high motility. The CAFs also produced another protein, Collagen III, which could stick the cancer cells and CAFs together. Because of the high motility of CAFs, cancer cells migrated with CAFs into other organs. Therefore, targeting the signal axis of CAFs and TAMs could become a new strategy in PDAC treatment.

In **Paper V**, we presented a link between the biological clock disruption and cancer migration. Studies showed that patients with cancer suffered from sleep disorder, which induced biological clock disruption in bodies. We used a mouse genetic model with deletion of the biological clock key gene Bmal1 to mimic the biological clock disruption condition. We found that cancer grew aggressively in these mice. After looking at the histology, we found that the key players are CAFs. Bmal1 deletion results in its under protein PAI-1 low expression. Low PAI-1 could not suppress tPA and uPA, which induced plasminogen converts into plasmin. The accumulation of plasmin converts latent TGF-β to become active. The active TGF-β then trigger CAFs to multiply and spread. Cancer cells are spread with CAFs to remote organs, such as to lung and liver. Therefore, blocking TGF-β and maintaining biological clock health can become the approach to treat cancer.

In summary, the works in this thesis uncover important players and their roles in the TME, which lay the ground for the development of novel pharmaceutical approaches in cancer therapy.

ABSTRACT

In the tumor microenvironment (TME), different cell components crosstalk with each other through various growth factors, cytokines, chemokines, and enzymes. They offered crucial survival signals for tumor cell proliferation and metastasis. Data presented in this thesis demonstrate approaches to target the stroma of the TME, which provided a novel paradigm for cancer therapy. It involves the conception of overcoming the resistance of conventional therapy, cut-out cancer cell energy support, and interrupting invasive assistance of cancer cells. We specifically focused on these two aspects: first, to resolve antiangiogenic drugs (AAD) resistance in those cancers with a lipid-rich environment or high FGF expression. In addition, to manipulate the brown adipose tissue (BAT) into mega BAT can be an approach in cancer therapy (Paper I-III). Second, we revealed the roles of stromal cells, including the cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs), in pancreatic ductal adenocarcinoma (PDAC) (Paper IV); and the role of CAFs in the cancer hosts with disturbed circadian rhythm (CR) (Paper V).

In **Paper I**, we revealed the mechanism of AAD drug resistance in tumors surrounded by adipose tissue or lipid-rich environment, for example, colorectal cancer (CRC), PDAC and hepatocellular carcinoma (HCC) in the steatotic liver. Anti-VEGF-based AAD failed to reduce tumor size but triggered vessel regression in tumor tissues, leading to severe hypoxia in the TME. Hypoxia resulted in tumor metabolic reprogramming from glucose-based metabolism to free fatty acids (FFAs)-based metabolism. FFAs provided energy support to tumor cells, leading to accelerate tumor cell proliferation. Inhibition of FFA transporter reversed AAD resistance in anti-tumor effect. Our data suggested a therapeutic approach to reverse the AAD resistance in tumors with a lipid-rich environment.

Our previous study revealed the cold exposure and other β3-adrenorecptor stimuli induced the brown adipose tissue (BAT) activation by increasing the non-shivering thermogenesis (NST). The activated BAT decreased blood glucose and impeded glycolysis-based metabolism in cancer cells could suppress tumor growth. In **Paper II**, we addressed an approach to enlarge BAT into a mega-size BAT (megaBAT) in adult animals. In BAT, the differentiation of certain progenitor cells is controlled by the platelet-derived growth factor receptor α (PDGFRα). Using pharmacological approaches and genetic deletion, we downregulated the PDGFRα in BAT progenitor cells and promoted progenitor cells differentiation into functional brown adipocytes. We found a specific microRNA to target the PDGFRα signaling *in vivo*. The whole BAT tissue mass was markedly increased after PDGFRα inhibition owing to the increase of brown adipocyte numbers. We found that the obese mice with megaBAT under cold exposure showed improvement in blood glucose level, insulin tolerance, and blood lipid level. Histological analysis showed that the steatotic livers were markedly reversed in obese mice with megaBAT. The megaBAT could become a therapeutic approach to treat cancer and metabolic diseases.

We previously reported that fibroblast growth factor 2 (FGF-2) as one of the angiogenic factors contributed to tumor vessel remodeling by recruiting NG2 positive pericytes onto tumor vessels through the PDGFRβ signaling. Therefore, monotherapy with anti-VEGF or anti-PDGFR had

become resistant in tumors with high FGF-2 expression. So far, there are no potent anti-FGF drugs available. In **Paper III**, we found combination therapy with anti-VEGF and anti-PDGFRβ showed superior anti-tumor effects in high FGF-2 tumors. Anti-PDGFRβ treatment suppressed pericyte recruitment, and anti-VEGF precisely targeted tumor vessels. With this study, we provided a new paradigm for resolving AAD resistance by targeting FGF-2 off-target signaling, VEGF and PDGF in cancer therapy.

In **Paper IV**, we identified the CAFs-TAMs crosstalk through the IL-33-ST2-CXCL3-CXCR2 axis in PDAC and triggered cancer cell metastasis. Mouse and human PDAC samples under unbiased genomic-wide profiling analysis and genetic and pharmacological gain/loss-of-function experiments demonstrated a high level of IL-33 expression. IL-33 bound to its receptor ST2 on the TAMs and induced TAMs infiltration. Transcriptomic analysis identified IL-33-ST2 induced high CXCL3 expression, which was produced by TMAs. CXCL3 bound to its receptors CXCR2 on CAFs induced CAFs-myoCAF transition and cell proliferation. CAFs transited to myoCAFs had a high expression of collagen III, which induced the formation of tumor cells and myofibroblast clusters. Tumor cells increased metastasis under this tumor-myofibroblasts clusters. Pharmacological targeting of this pathway would provide a potential therapeutic strategy for treating PDAC.

Paper V we presented the link between CR disruption and tumor metastasis. We explored CR disruption by using a genetic model of *Bmal1* gene knockout (KO) mice. Various types of tumors in *Bmal1* KO mice presented high growth speed with an elevated expression of myofibroblast markers. Unbiased genomic-wide profiling using the stromal vascular fraction (SVF) from the tumors of *Bmal1* KO mice demonstrated a downregulated expression of the plasminogen activator inhibitor (*PAI-1*) gene. The BMAL1 protein directly regulated *PAI-1* gene transcription. Lacking BMAL1 resulted in low PAI-1 expression, which continuously removed the inhibition of downstream proteins, including tissue plasmin activator (tPA) and urokinase (uPA). The tPA and uPA accumulation transformed plasminogen into plasmin, which converted the latent TGF-β into active form. The active TGF-β contributed to the CAFs transition into myofibroblasts, which induced tumor tissue mass expansion and increased metastasis. Inhibition of TGF-β in tumors with CR disruption or maintenance of CR homeostasis could be a therapeutic approach to tumor therapy.

Collectively, the works in this thesis uncover important roles of stromal cellular components in the TME, which lay the ground for the development of novel pharmaceutical approaches in cancer therapy.

LIST OF SCIENTIFIC PAPERS

I. H. Iwamoto, M. Abe, Y. Yang, D. Cui, T. Seki, M. Nakamura, K. Hosaka, S. Lim, **J. Wu**, X. He, X. Sun, Y. Lu, Q. Zhou, W. Shi, T. Torimura, G. Nie, Q. Li and Y. Cao (2018). "Cancer Lipid Metabolism Confers Antiangiogenic Drug Resistance." Cell Metabolism 28 (1): 104-117 e105.

II. Q. Du*, **J. Wu***, C. Fischer, T. Seki, X. Jing, J. Gao, X. He, K. Hosaka, L. Tong, A. Yasue, M. Miyake, M. Sobajima, S. Oyadomari, X. Sun, Y. Yang, Q. Zhou, M. Ge, W. Tao, S. Yao and Y. Cao (2022). "Generation of mega brown adipose tissue in adults by controlling brown adipocyte differentiation in vivo." Proceedings of the National Academy of Sciences 119 (40): e2203307119.

III. K. Hosaka, Y. Yang, T. Seki, Q. Du, X. Jing, X. He, **J. Wu**, Y. Zhang, H. Morikawa, M. Nakamura, M. Scherzer, X. Sun, Y. Xu, T. Cheng, X. Li, X. Liu, Q. Li, Y. Liu, A. Hong, Y. Chen and Y. Cao (2020). "Therapeutic paradigm of dual targeting VEGF and PDGF for effectively treating FGF-2 off-target tumors." Nature Communication 11 (1):3704.

IV. X. Sun, X. He, Y. Zhang, K. Hosaka, P. Andersson, J. Wu, **J. Wu**, X. Jing, Q. Du, X. Hui, B. Ding, Z. Guo, A. Hong, X. Liu, Y. Wang, Q. Ji, R. Beyaert, Y. Yang, Q. Li and Y. Cao (2021). "Inflammatory cell-derived CXCL3 promotes pancreatic cancer metastasis through a novel myofibroblast-hijacked cancer escape mechanism." Gut. doi:10.1136/gutjnl-2020-322744 (2021)

V. **J. Wu***, X. Jing*, Q. Du, X. Sun, J. Gao, X. He, K. Hosaka, C. Zhao, W. Tao, G. A. FtzGerald, Y. Yang, L. Jensen and Y. Cao. "Disruption of circadian rhythm promotes cancer metastasis through the BMAL1-PAI-1-TGF-β-myoCAF signaling mechanism" (manuscript)

*Equal contribution

SCIENTIFIC PAPERS NOT INCLUDED IN THE THESIS

I. S. Li, W. Li, J. Yuan, P. Bullova, **J. Wu**, X. Zhang, Y. Liu, M. Plescher, J. Rodriguez, O. C. Bedoya-Reina, P. R. Jannig, P. Valente-Silva, M. Yu, M. A. Henriksson, R. A. Zubarev, A. Smed-Sörensen, C. K. Suzuki, J. L. Ruas, J. Holmberg, C. Larsson, C. Christofer Juhlin, A. von Kriegsheim, Y. Cao and S. Schlisio (2022). "Impaired oxygen-sensitive regulation of mitochondrial biogenesis within the von Hippel-Lindau syndrome." Nature Metabolism **4**(6): 739-758.

II. X. Jing, **J. Wu**, C. Dong, J. Gao, T. Seki, C. Kim, E. Urgard, K. Hosaka, Y. Yang, S. Long, P. Huang, J. Zheng, L. Szekely, Y. Zhang, W. Tao, J. Coquet, M. Ge, Y. Chen, M. Adner and Y. Cao. (2022). COVID-19 instigates adipose browning and atrophy through VEGF in small mammals. Nature Metabolism.

III. L. Szekely, B. Bozoky, M. Bendek, M. Ostad, P. Lavignasse, L. Haag, **J. Wu**, X. Jing, S. Gupta, E. Saccon, A. Sonnerborg, Y. Cao, M. Bjornstedt and A. Szakos (2021). "Pulmonary stromal expansion and intra-alveolar coagulation are primary causes of COVID-19 death." Heliyon 7 (5): e07134.

CONTENTS

LIST OF ABBREVIATIONS

TME	Tumor microenvironment
ECM	Extracellular matrix
CAF	Cancer-associated fibroblast
TAM	Tumor-associated macrophage
EMT	Epithelial-mesenchymal transition
CAA	Cancer-associated adipocyte
VEGF	Vascular endothelial growth factor
CRC	Colorectal cancer
NSCLC	Non-small cell lung cancer
PD-L1	Programming death ligand 1
PD-1	Programming death 1
OS	Overall survival
FGF-2	Platelet-derived growth factor receptor
EC	Endothelial cell
HCC	Hepatocellular carcinoma
AAD	Antiangiogenic drug
α-SMA	α-smooth muscle actin
PDGFR	Platelet-derived growth factor receptor
mCAF	myofibroblast
CLEC3B	C-type lectin domain family 3 member B
iCAF	Inflammatory CAF
CAV1	Caveolin 1
CCL	Chemokine (C-C motif) ligand
CXCL	C-X-C motif chemokine
CSF1	Colony-stimulating factor 1
HGF	Hepatocyte growth factor
IGF1	Insulin-like growth factor 1
IL	Interleukin
LIF	Leukemia inhibitory factor
PGE_2	Prostaglandin E_2

CAR-T cell	Chimeric antigen receptor T cell
FFA	Free fatty acid
FATP	Fatty acid transport protein
FABPpm	Plasma membrane fatty acid binding protein
LD	Lipid droplet
NADPH	Nicotinamide adenine dinucleotide phosphate
GLS1	Glutaminase 1
TAG	Triacylglycerol
PA	Phosphatidic acid
MUFA	Monounsaturated fatty acid
SCD	Stearoyl-CoA desaturase 1
FASN	Fatty acid synthase
ACLY	ATP-citrate lase
ACSS2	Acyl-CoA synthetase short-chain family member 2
APC	Antigen presenting cell
LPS	Lipopolysaccharide
TLR-4	Toll-like receptor 4
NF-κB	Nuclear factor kappa B
TNF-α	Tumor necrosis factor
MHC-II	Major histocompatibility complex class II
BMDM	Bone marrow-derived monocyte
CSF-1	Colony-stimulate factor 1
MIP-1α	Macrophage inflammatory protein 1 alpha
MIF	Macrophage migration inhibition factor
MDSC	Myeloid-derived suppressor cell
TRM	Tissue-resident macrophage
CR	Circadian rhythm
SCN	Suprachiasmatic nucleus
BMAL1	Brain and muscle ARNT-Like 1
CLOCK	Circadian locomotor output cycles kaput
CRY	Cryptochromes

PER	Period
Klf4	Kruppel-like factor 4
NE	Noradrenaline
REM	Rapid eye movement
MYF5	Myogenic factor 5
SCA-1	Stem cell antigen 1
UCP1	Uncoupling protein 1
COX4	Cytochrome c oxidase subunit 4
NST	Non-shivering thermogenesis

1 INTRODUCTION

The tumor microenvironment (TME) refers to the surrounding environment of cancer cells. It is formed by different cellular components, various growth factors, cytokines, and extracellular matrix (ECM) [1,2]. In the TME, there are various cellular components, including cancer-associated fibroblast (CAF), tumor-associated macrophage [3], vascular endothelial cell (EC), pericyte, immune cell, as well as adipocyte (Fig 1). The TME plays an important role in acquiring and maintaining the hallmarks of cancer by the highly dense microvessels, quiescent immune cells, affluent protein growth factors, adhesive ECM and, as well as metabolic reprogramming under hypoxia [4]. All these TME components create an appropriate soil for cancer cells, and the cellular components of the TME also participate in tumor mass expansion. However, along with the tumor expansion, the living environment of cancer cells continually deteriorates with the increased competition in acquiring nutrition and oxygen stress. Therefore, cancer cells would seek opportunities for invasion and metastasis under the trigger of intrinsic genetic changes and extrinsic environment stress [5]. During cancer cell metastasis, the components of the TME continue to provide their support to cancer cells. For example, the TAMs and CAFs contributed to the epithelial-mesenchymal transition (EMT) at the primary sites, facilitating cancer cells to depart from neighbor cells and attain a mobile and invasive phenotype [6].

In the TME, the stromal cellular components are particularly referring the CAF, EC and pericyte [7]. Later studies revealed adipocytes were also differentiated from stromal progenitor cells [8]. The cancer-associated adipocytes (CAAs) presented in several types of tumors, including breast and colorectal cancer [9]. The cancer cells secrete assorted growth factors and chemokines and cytokines to the TME. Under the effect of cancer cells, the stromal cellular components crosstalk with each other, acting as tumor-promoting accomplice. For example, studies found the CAFs promote cancer fibrosis, which is believed to occur under the mechanism of the uncontrolled wound repair process and peculiar inflammation [10], and cancer fibrosis is one of the major problems of treatment unresponsive in some types of tumors [7]. In clinic, the low cancer-stromal ratio is representing as a poor prognosis marker [11]. Also, the stromal cells distribute variously in the TME have contributed to form a heterogeneous environment in tumor tissue. Cancer heterogeneity is another reason result in ineffective therapeutic response [12]. Moreover, recent studies revealed the interplay between tumor-associated stromal components and immune cells contribute to the TME via dysregulating the immune response. For example, studies showed CAFs promoted TAM recruitment by releasing CXCL12 and CXCL14 [13]. Apart from the impacts of cancer cell, some other factors in the TME are indicated have highly effects on the stromal cells and have roles in tumor-promoting. For example, the TME hypoxia is markedly relevant to tumor angiogenesis [14]. Different nutrition and diets were proved to alleviate cancer progression or treatment effects in patients [15,16]. Studies showed that the circadian rhythm (CR) core genes disruption resulted in stromal cells proliferation and led to tumor metastasis [17].

Targeting cancer cells therapies, including chemotherapy and radiation therapy, have emerged for half a century. Although they are still the major approaches in the clinic, these therapies have shown low consistent efficiency in various cancer. Also, they are notorious for the side effects on patients, resulting in poor life quality and outcome [18]. In the last two decades, tyrosine kinase inhibitors (TKIs) emerged as an approach to target specific signaling in cancer cells [19]. The TKIs have become an effective approach for some kinds of cancers, which previously were believed difficult to treat, including non-small cell lung cancer (NSCLC) and melanoma [20 21]. However, the TKIs becoming resistant to secondary mutation has resulted in shortening treatment efficacy.

Because the TME contributes to maintain the hallmarks of cancer in various aspects, targeting the TME has become an ideal strategy in cancer therapy. One of the most famous examples is targeting angiogenic therapy in the late 1990s [22]. Bevacizumab, as the inhibitor of vascular endothelial growth factor (VEGF), combined with chemotherapies, has been proven significant effects on colorectal cancer (CRC) and NSCLC [23]. Another example is the first-line immune checkpoint therapy of inhibiting programming death ligand-1 (PD-L1) and its receptors PD-1, which markedly improved overall survivals (OS) in the late stage of melanoma patients [24]. Recently, several therapeutic approaches target stromal cellular components in the TME have been conducted into clinical trials, for example, inhibiting therapies of CAFs and TAMs [25], indicating treatments targeting on the TME are promising.

In this thesis, I demonstrate different stromal cellular components and their function in the TME. By assembling our research results, I provided and discussed potential therapeutic approaches for cancer therapy.

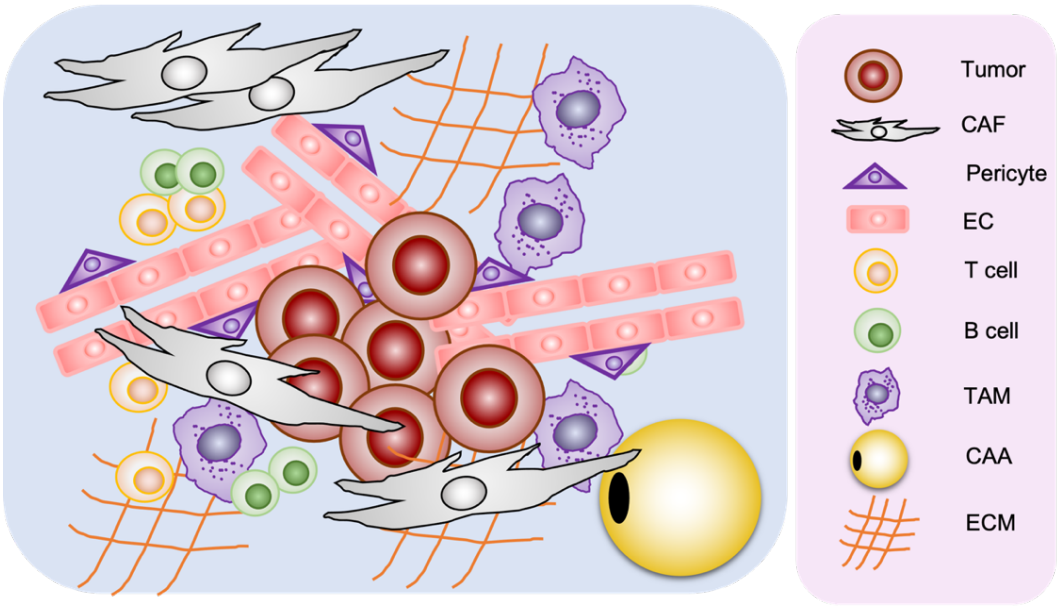

Fig. 1 Schematic illustration of the components of the TME. Abbreviations: CAF, cancer-associated fibroblast; EC, endothelium cell; TAM, tumor-associated macrophage; CAA, cancer-associated adipocyte; ECM, extracellular matrix.

2 LITERATURE REVIEW

In order to target the stromal cellular components in the TME, we need to understand their roles in cancer growth and metastasis. In this literature review, I introduced the roles of different cellular components in the stroma of cancer and various approaches of targeting stromal components. Also, I introduced the emerging link between circadian rhythm and stromal components in cancer.

2.1 THE ENDOTHELIAL CELL, PERICYTE AND TUMOR ANGIOGENESIS

Date back to the 1970s, Dr. Judah Folkman firstly described solid cancer grow is depending on angiogenesis [26]. Pathological research showed that, unlike healthy blood vessels, the tumor vessel networks are disorganized, unevenly distributed, and dilated with highly permeable and lack of pericyte coverage (Fig 2) [27]. With these characteristics, the vessel capacity of nutrient and catabolite transportation is insufficient, which aggravates hypoxia in tumor microenvironment [28] and triggers more growth factors be produced to promote tumor cell proliferation and metastasis [29,30]. Dr. Folkman assumed tumor cells secreted some factors to induce angiogenesis to support tumor growth [26], and he and his team confirmed their hypothesis by isolating the first angiogenesis factor, which was named as tumor angiogenesis factor [31] and it had been identified as fibroblast growth factor (FGF) 2. Tumor cells produce angiogenic factors, such as FGF-2 and later the most well-known factor VEGF, which stimulate the quiescent endothelial cells (ECs) in capillaries to sprout out more capillaries [29]. The spearheading in vascular sprouts are the endothelial tip cells [32]. With a highly expression of VEGF receptors 2, the tip cells receive strong stimulation of VEGF [33]. The tip cells are characterized with long and dynamic filopodia, which results in migratory behavior of tip cells. Following the tip cells, the endothelial stalk cells show fewer filopodia, but highly proliferative manner. The stalk cells establish the cell junction and stabilize the new sprout. They also form the lumen of nascent vascular [32]. Apart from ECs, the pericytes and vascular smooth muscle cells (vSMC) located in the interface between ECs and the surrounding tissue also contribute to the angiogenesis process [34]. Researchers found pericytes could sense the angiogenic stimuli and contact with ECs to integrate the signals along the vessel length [34].

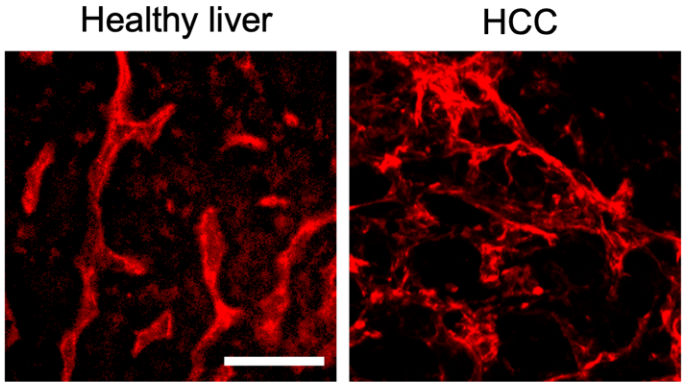

Fig. 2 The comparison between healthy liver vessels and hepatocellular carcinoma (HCC) vessels. In HCC, the vessel network is disorganized and uneven with high permeability. Bar = 50 μm.

Dr. Folkman proposed targeting angiogenic factors to impair tumor growth, which later opened the field of anti-angiogenesis in tumor therapy [26]. So far, direct and indirect inhibitors for angiogenesis have been developed. Direct inhibitors are used to target ECs. The Direct inhibitors, such as endostatin, thalidomide, cell cycle inhibitors TNP-470 and integrin inhibitors, have gained some success in vitro and animal-bearing tumor models. A few of them also showed efficiency in benign tumors. The indirect inhibitors mainly target to the function of angiogenesis. Bevacizumab, the first anti-VEGF drug approved by FDA, had shown efficacy in patients under combination therapy with cytotoxic chemotherapy in certain tumors, for instance, colorectal, renal and lung cancer [35]. However, even bevacizumab combined with chemotherapy had obtained a compelling effect in preclinical studies, monotherapy with bevacizumab did not significantly anti-tumor and prolong OS in patients with metastatic disease [29,36].

To explain why an anti-VEGF-based antiangiogenic drug (AAD) monotherapy in the clinic does not obtain sufficient inhibitory effects, on one side, some researchers claimed mouse tumor has different morphology from human tumors, resulting in a in lower therapeutic effect. On the other hand, researchers found drug resistance may be the main reason [37]. Several drug resistance mechanisms of AAD have been revealed. For instance, studies found AAD initially caused tumor vessel regression and leaded to hypoxia in the TME. Hypoxia transcriptionally induced VEGF expression to promote tumor growth and metastasis [38]. In addition, tumor cells produced other angiogenic factors upon anti-VEGF-based treatment to compensate for the effect of VEGF, which including the FGF, platelet-derived growth factors (PDGFs) and angiopoietin [37]. Researcher found tumor cells could use vessels in healthy tissue to transport nutrient and catabolic products. This modulation with AAD was named as vessel co-option [38]. Apart from using healthy vessel in adjacent tissue, tumor cells had been reported to recruit other cells, for instance, immune cells, fibroblasts, and progenitor cells, to form shields and block the drugs targeting on ECs [38].

So far, the mechanism of AAD resistance has not been fully understood, but hitting these resistant points may reverse AAD's function in tumor therapy.

2.2 THE CANCER-ASSOCIATED FIBROBLAST

Cancer-associated fibroblasts (CAFs) have been defined as fibroblasts growing inside tumor tissue and they have abundant accumulation in the stroma of various tumors [39]. Different from healthy fibroblasts, CAFs showed tumor-promoting phenotypes with higher proliferation rate and motility. There are various possible origins of CAFs (Fig 3): 1) they are resident fibroblasts [40]. A study used the GFP locating fibroblast co-staining with the collagen I and α-smooth muscle actin (α-SMA) indicated CAFs are resident in the tumor original tissues, which was activated by epigenetic modulation of tumor cells. 2) They are recruited by cancer cells. By analyzing the CAFs from patients with gender-mismatched bone marrow transplantation, researchers found CAFs have myeloid cells expression [39,41]. They considered these CAFs were recruited from bone marrow by the cancer cells. 3) CAFs were transited from epithelial cells. Some researchers claimed that EMT could contribute to the formation of CAFs [40]. 4) Other

stromal cells differentiation. Studies found that pericytes and endothelial cell in the tumor tissue could differentiate into CAFs under the stimulation of cancer cells [39]. A study used the single-cell sequencing of 768 transcriptomes of mesenchymal cells from mice model with breast cancer had revealed the subpopulation of CAFs origins from peri-vascular niche, mammary fat pad and the transformed epithelium [42].

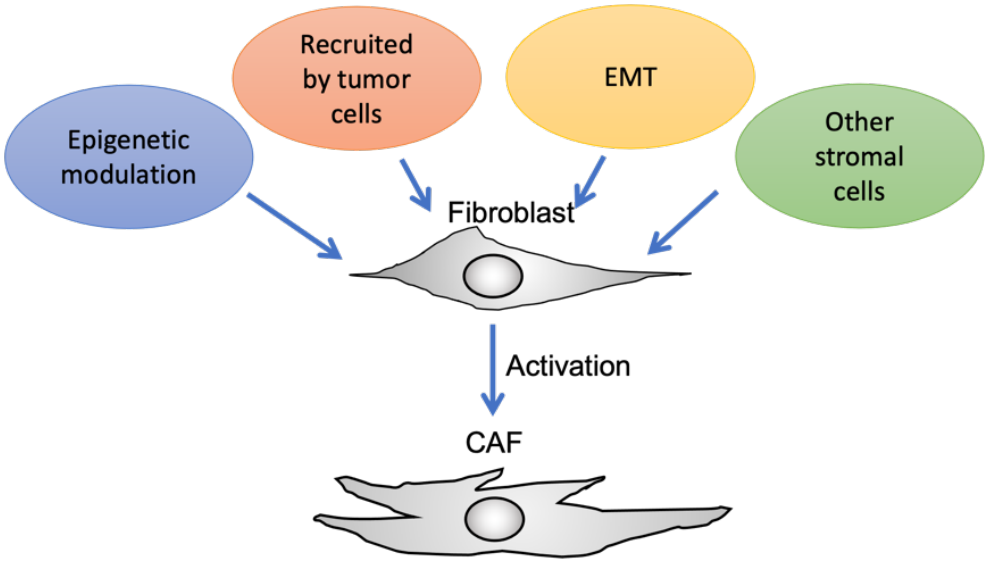

Fig. 3 Schematic illustration of various mechanisms involved in CAFs origins.

There are various markers have been used to identify CAFs, including α-SMA, platelet-derived growth factor receptor α (PDGFRα) and β, vimentin, desmin, fibroblast specific protein 1 (FSP1), fibroblast activation protein (FAP), and discoidin domain-containing receptor 2 (DDR2) [43]. By using single-cell sequencing and immunofluorescent co-staining in CAFs, CAFs showed heterogeneity in expression [43,44]. This heterogeneity indicates CAFs subpopulation distribution and tumor-promoting functions. For example, studies found CAFs with α-SMA expression showed myofibroblast (mCAFs) phenotypes in many cancers [45]. CAFs with C-type lectin domain family 3 member B (CLEC3B) and collagen alpha -1 (XIV) expression indicate inflammatory (iCAFs) phenotypes. The mCAFs are in proximity to cancer cells, but iCAFs are more distant. Some studies used isolated CAFs from patients' renal cell carcinoma (RCC) samples to stain with PDGFRβ, revealing CAFs with PDGFRβ high expression results in poor prognosis but CAFs with higher PDGFRα expression showed better prognosis [46].

The CAFs produce various growth factors and cytokines to form most of the ECM. These growth factors and cytokines directly or indirectly support tumor growth [39]. Studies found the CAFs secrete matrix metallopeptidase 9 (MMP9), which facilitates TGF-β activation [47]. Elevated active TGF-β in the TME induced tumor cells proliferation and CAFs transition into mCAFs. Some researchers found TGF-β accumulation could also enhance glycolysis in CAFs to promote CAFs proliferation [44]. Since active TGF-β attenuated Caveolin 1 (CAV1) expression in the TME. As a tumor suppressor gene, CAV1 negatively regulates Ras/MAPK cascade and joins cell cycle progression [48]. Diminished CAV1 expression resulted in increasing

glycolysis in both tumor and CAFs. Apart from TGF-β, another signaling pathways have been revealed in tumor and CAFs communication, including chemokine (C-C motif) ligand 2 (CCL2), CCL5, C-X-C motif chemokine 5 (CXCL5), CXCL9, CXCL10, CXCL12, colony-stimulating factor 1 (CSF1), Hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF1), PDGF, VEGF, interleukin family proteins (IL-1, IL-4, IL-6, IL-8, IL-10), leukemia inhibitory factor [49] [49], and prostaglandin E_2 (PGE_2) [43]. As the main resource of ECM, CAFs contribute to the formation of tumor fibrosis. In healthy conditions, fibrosis is a process of healing wounds. However, tumor cells trigger CAFs proliferation to form an environment like a wound "never heal" [50]. Eventually, the accumulation of fibronectin, collagens, and CAFs in the TME become fibrosis, which act as a shield for cancer cells, preventing drug treatment and facilitating metastasis [22]. Data from some pre-clinic studies also showed CAFs' tumor-promoting function by immune suppression [43]. A study showed CAFs in HCC induced chemotaxis of neutrophils and impaired the function of T cells through immune checkpoint PD-1/PD-L1 signaling pathway [51]. Some studies indicated that CAFs specifically expressed FAP subgroups contributed to immune suppression by producing IL-6 and CXCL12 [52].

Because CAFs' multiple functions in the TME, targeting CAFs and their secretion can become potential approaches in cancer therapy. Previously, an anti-FAP mono neutralized antibody sibrotuzumab had been tested in a phrase I dose escalation trial in advanced colon and lung cancer patients [53]. But this drug was failed in a phrase II trial [54]. Later, in some pre-clinical studies, genetic FAP deletion in CAFs became an approach to alter sensitivity to immunotherapies. For example, a study showed FAP^+ CAFs depletion induced tumor cell necrosis associated with $CD8^+$ T cell infiltration in PDAC [55]. Meanwhile, another study conducted FAP^+ CAFs elimination by DNA vaccines served as a complement in the chimeric antigen receptor T cells (CAR-T) treatment since eliminating FAP^+ CAFs increased $CD8^+$ T cells killing CAFs in tumors and assisted intratumor intake chemotherapeutic drugs [55]. Other similar markers, for example, depletion of α-SMA in CAFs, have also been used in pre-clinic studies. However, since there are no absolute CAFs markers, identifying the subpopulations of CAFs becomes necessary for anti-CAFs therapies. The single-cell sequencing approach and transcriptomic analysis for CAFs subpopulation identification can be applied in the clinic as a personalized analysis before treatment.

Also, other strategies in anti-CAFs therapy have emerged by targeting the secretion products from CAFs, for example, the collagens and MMPs. Some studies showed that anti-collagen I antibodies had efficiency in the mouse tumor model of prostate cancer, pancreatic cancer and lung cancer [54]. MMP antibodies had been shown to decrease tumor mass and metastasis in the mouse model. Recently, a CXCR4 inhibitor had shown to improve myeloid cell recovery rate in myeloid leukemia by phase I-II study, which indicating anti-CAFs secretion cytokines could also become assisting approach in immunotherapies. Other research tried to cut down the tumor cell-CAFs communications by using TGF-β inhibitor combined with PD-L1 therapy in metastatic pancreatic cancer. However, the study did not bring significant benefits to patients [56].

Although targeting CAF and its downstream signaling and secretion products can be a strategy of tumor therapy, currently there are no effective direct inhibitors due to the heterogeneity of CAFs. The combination therapies of targeting subpopulations of CAFs with immune checkpoint inhibitors and CAR-T therapies may be prospective in the clinic.

2.3 THE CANCER-ASSOCIATED ADIPOCYTE

According to the data from WHO, obesity is associated with tumor incident rate in various types of tumors, especially those types of tumors grow in organs with or adjacent to adipose tissue (AT) [57]. For example, breast cancer, stomach cancer, colorectal cancer, renal cancer, and HCC [58]. Studies indicated visceral fat accumulation, which is common with aging, was specifically related to cancer progression [58]. The adipocytes located adjacent to tumor cells are referred to as CAAs [58]. Mechanistic studies showed that CAAs contribute to tumor growth by providing free fatty acids (FFAs), formation of cell membranes and other organelles, chronic inflammation, circulating steroid hormones changing and insulin intolerant, as well as a protease for cancer invasion [57,58].

The fatty acids (FAs) are the formation of several lipid species, including triglycerides, phospholipids, and sphingolipids. They are distinguished by various compositions of a carboxylic acid group and a hydrocarbon chain of different lengths and various degrees of saturation [59]. Lipolysis releases FAs into bloodstream and these FAs are called free FAs (FFAs) [60]. Tumor cells obtain FFAs mainly through exogenous uptake and *de novo* lipogenesis (Fig 4) [59,61]. Exogenous FFAs uptake requires a transporters-dependent manner in tumor cells. The specific FFAs transporters include CD36, fatty acid transport protein family (FATPs) and plasma membrane fatty acid binding proteins (FABPpm) [59]. Extracellular FFAs are transported through these transporters into tumor cells, and later participate in storing as lipid droplets (LDs), which could be used in acetyl-CoA or nicotinamide adenine dinucleotide phosphate (NADPH) process through the β-oxidation pathway. The *de novo* lipogenesis in term of tumor cells produces LDs by glucose and glutamine transition. The uptake of FFAs and *de novo* lipogenesis are regulated by mutated oncogenes in tumor cells. Studies found *KRAS* gene mutation induced glycolysis in PDAC. Also, the amplification of MYC drove to an over activation of mitochondrial glutaminase 1 (GLS1) and glutamine transporter SLC1A5 [59]. Meanwhile, the stress of TME, for example, hypoxia, also contributes to metabolism reprogramming through the FFAs uptake and lipogenesis [62].

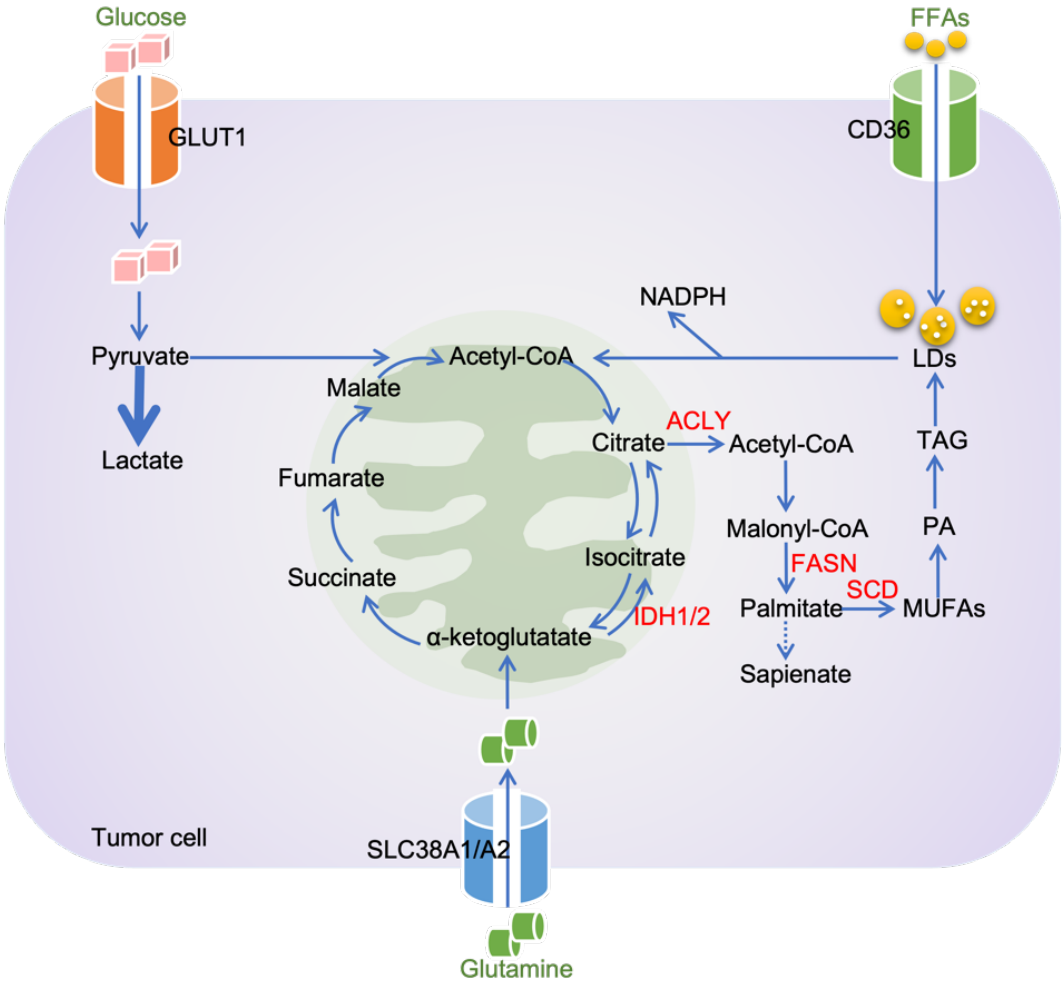

Fig 4. Schematic illustration of FFAs uptake and *de novo* lipogenesis in tumor cell. Abbreviation: FFAs, free fatty acid; LDs, lipid droplets; TAG, triacylglycerol; PA, phosphatidic acid; MUFAs, monounsaturated fatty acids; SCD, stearoyl-CoA desaturase-1; FASN, fatty acid synthase; ACLY, ATP-citrate lyase; NADPH, nicotinamide adenine dinucleotide phosphate; IDH1/2, Isocitrate dehydrogenase 1 and 2.

Because of the function of FFAs in supporting tumor growth, inhibiting FFAs uptake and *de novo* lipolysis become an approach in tumor therapy. Pre-clinic studies and clinical trials had applied various drugs targeting on FFAs metabolic reprogramming, including inhibitors of fatty acid synthase (FASN), ATP-citrate lyase (ACLY), acyl-CoA synthetase short-chain family member 2 (ACSS2) and stearoyl-CoA desaturases-1 (SCD) [59]. The mechanism of these inhibitors is depending on the limit-enzyme effects of FFAs transportation and the acyl-CoA. They had demonstrated significant effects on tumor suppression. However, despite the good efficacies, side effects such as inducing body weight loss were dramatically. Since tumor cells share the same metabolic pathways with healthy cells, inhibiting FFAs *de novo* lipogenesis directly affected whole body adipose tissue metabolism [59]. Some of them had been improved in the second-generation drugs. The effects of these new drugs deserve further investigation.

2.4 THE TUMOR-ASSOCIATED MACROPHAGE

TAMs are referred to the monocytes infiltrated into tumor tissue. Originally, macrophages are derived from bone marrow-derived monocytes [39]. Promonocytes from bone marrow

differentiate into monocytes, and monocytes migrate into blood strain. Later, they migrate into tissue and become macrophages. In healthy conditions, macrophages distribute in different tissue to serve different functions: In skin or lung, they serve as Langerhans cells to clear up pathogens and improve local inflammation; Kupffer cells are macrophages in the liver that assistant to clear up pathogenic and catabolic materials; the population located in spleen would handle erythrocyte recycling [31]. All these macrophages are antigen-presenting cells (APCs), and their activation is named as polarization. First, macrophages would recognize the pathogen-associated molecular patterns, such as lipopolysaccharide (LPS), lectins, protein, oligonucleotides, and polysaccharides of antigens [63]. The surface receptor Toll-like receptor 4 (TLR-4) on macrophages would interact with the pathogen-associated molecular to active transcription factors, for instance, nuclear factor kappa B (NF-κB) to raise inflammation. After recognizing the pathogen-associated molecular, macrophages polarize into classical activated M1 macrophages and secret multiple cytokines, including interleukin-1β (IL-1 β), IL-6 and tumor necrosis factor (TNF-α), to recruit more macrophages and active lymphocytes to join the immune reaction to kill pathogens [31]. Also, macrophages express major histocompatibility complex (MHC) class II on the membranes and present antigens to lymphocytes [63]. In a wound scenario, killing and damage are not the purpose of macrophages. Instead, they would respond to cytokines like IL-4 and IL-13 from damaged cells and polarize into alternatively activated macrophages (M2s) to anti-inflammation and heal the tissue by activating angiogenesis downstream pathway and basement membrane remodeling [31]. The polarization could be classified into pro- and anti-inflammatory [64].

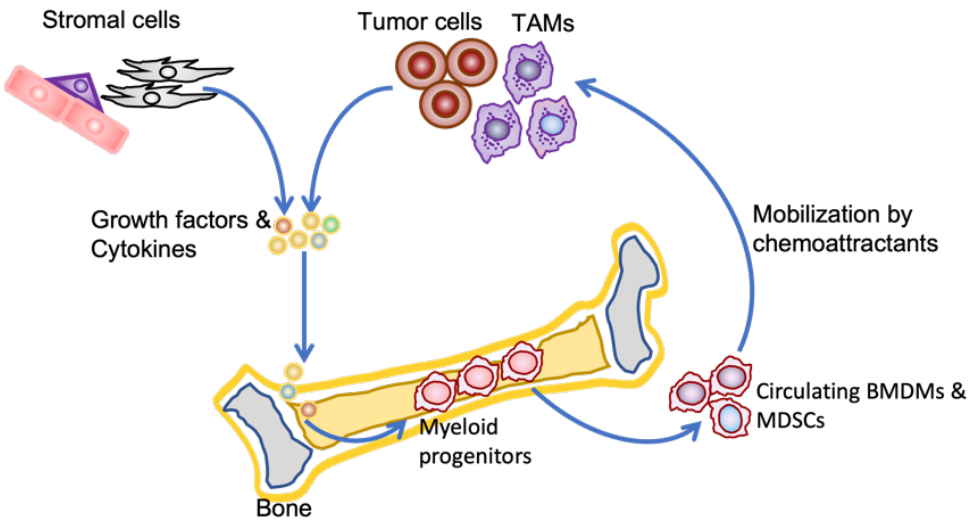

Fig 5. Schematic illustration of TAM recruitment. The tumor cells and stromal cells released growth factors and cytokines to the bone marrow and stimulated myeloid progenitor cells generation. The BMDMs and MDSCs moved into circulation and were recruited to the TME. BMDMs, bone marrow-derived macrophages; MDSCs, myeloid-derived suppressor cell.

In tumors, the circulating bone marrow-derived monocytes (BMDMs) are considered as the main resource of TAMs [65,66]. Multiple growth factors and cytokines, such as colony-stimulate factor 1 (CSF-1), CXCL2, interleukins family proteins, VEGF, macrophage inflammatory protein-1 alpha (MIP-1α) and macrophage migration inhibition factor (MIF) [65] were released

by tumor cells and stromal cells to alter myelopoiesis in the bone marrow, inducing the generation of immature myeloid cells with immunosuppressive abilities, for example, the myeloid-derived suppressor cells (MDSCs) and BMDMs (Fig 5). The MDSCs and BMDMs were recruited into tumor tissue and received further alteration [67]. Some other studies indicated the tissue-resident macrophages (TRMs) could also turn into TMAs. However, the TRMs and BMDMs showed distinguished expression profiling [65]. Under the continues stimulation of tumor cells, TAMs polarized into M1 and M2 phenotypes, and they served opposite functions by promoting and inhibiting tumor growth. Studies showed that M1 macrophages had the ability to kill tumor cells through their cytokines and immune cell activation. However, M2 macrophages produced a lot of angiogenesis factors and proteolytic enzymes would support tumor angiogenesis and invasion [31]. Some studies showed that during the early stage of tumorigenesis, there were more M1-like macrophages since it was the link between inflammation and tumorigenesis [68]. Most studies discussed TAM were likely referring to this M2 type macrophages that infiltrated in tumor. The TMAs also showed overexpression of PD-L1, which binded to the PD-1 on T cells and inhibits T cell activation and T cell killing. Some studies showed a subpopulation of TAMs expressed PD-1 as well. The increased expression level of PD-L1 in TAMs was correlated with a poor prognosis of advanced colorectal cancer. Other study found inhibition of PD-1/PD-L1 had an effect on immunodeficient mice with tumors. The PD-1/PD-L1 inhibition would increase the ability of TAMs to engulf tumor cells [67].

Because of the immunosuppression role of TAMs in the TME, targeting TAMs becomes an approach to alter the immune checkpoint effect and against tumor growth. Different strategies of inhibiting TAMs have been developed, which particularly on the formation of TAMs and their downstream signaling. For example, by inhibiting CCL2 could inhibit TAMs recruitment. Antibodies targeting on the CCL2 receptors (CCR2) have been combined with chemotherapy, and showed significant effects on decreasing tumor size and slowing tumor growth [65]. Studies used CSF receptor (CSF1R) inhibitor and its kinase inhibitor to suppress breast tumor growth and reverse chemotherapy resistance [65]. Combination therapy of AAD and anti-CCL2/CCR2 showed anti-tumor effects since AAD eliminated angiogenesis in tumors. Combination therapy of anti-TMA and anti-immune checkpoints therapies could reverse resistance of immune checkpoint therapy by increasing T cell killing effect [67,68].

2.5 THE EMERGING LINK BETWEEN TUMOR STROMA AND CIRCADIAN RHYTHM

The circadian rhythm (CR) system in most vertebrate and invertebrate organisms is generated by the shift of solar light and dark on the earth [69,70] [71]. This intrinsic system controls various physiological processes in the human body, including metabolism, neurological activity, immunity, endocrinology, and vascular biology [72]. Studies found that disruption of CR is one of the high risks of many diseases, including cardiovascular diseases, obesity, metabolic diseases and cancer [69,70,73].

In human, the CR molecular mechanism is under a cell-autonomous transcriptional autoregulator feedback loop [71]. When the human retina of eyes receives the natural light, a central peacemaker (core clock) is generated and maintained in the suprachiasmatic nucleus (SCN) of the hypothalamus in the brain [70,71]. So far, researchers found there are three interlocking feedback loops to control the core clock (Fig 6). In the core loop, the control genes are including circadian locomotor output cycles kaput (*CLOCK*) and brain and muscle ARNT-Like 1 (*BMAL1*) genes. The CLOCK and BMAL1 protein form as heterodimer and transcriptional control their downstream genes by targeting on the E-box of promoters [71]. The downstream genes in this feedback loop are including *Period* (*PER1*, *PER2*, and *PER3*) and *Cryptochromes* (*CRY1* and *CRY2*) genes. Their protein expression accumulated during the daytime and reached to the peak. The accumulated proteins react as the negative feedback to inhibit the transcription of *CLOCK* and *BMAL1* genes. The transcription of *CLOCK* and *BMAL1* decreased, and later the transcription of downstream genes decreased as well along with the light shifted to dark in the environment. This autoregulator feedback loop would function again as the sunlight rises on a new day. It oscillates with the light and dark in a 24-hour shifting [70,71]. There are two families of nuclear receptors, including the REV-ERBα/β and RAR-related orphan receptors α/β (RORA/B), to stabilize the core loop and regulate transcriptional output in a distinct phase. They directly target on the CLOCK-BMAL1, and antagonistically regulate *BMAL1* transcription by binding to the ROR/REV-ERB-response elements (RORE) to form a secondary loop. The third loop involves the repressor E4 promoter-binding protein 4 (E4BP4) and the proline and acidic amino acid-rich basic leucine ZIPper (PAR-bZIP) protein, which competitively binds to the D-box of the promoter of *RORA/B* genes and joined the core loop and secondary loop. Besides of the core clock network, the peripheral clock is distributed in the whole body to regulate peripheral organs physiology [71,74]. Inhibiting of the players in the CR feedback loop is considered as the CR disruption.

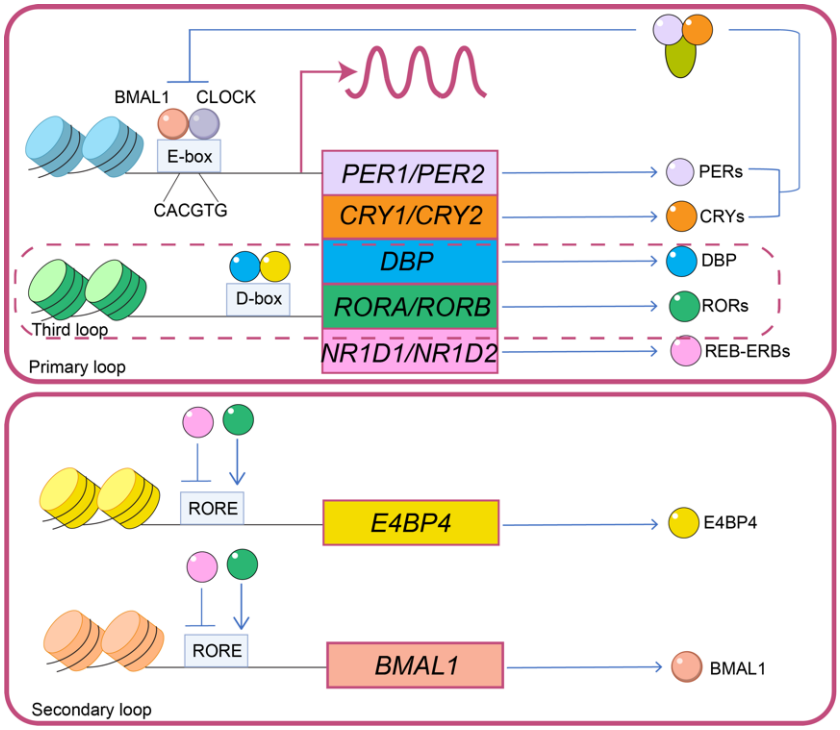

Fig 6. Schematic illustration of CR transcription loops. So far, there are three autoregulator feedback loops were found in the core clock. The first loop is controlled by the CLOCK and BMAL1 heterodimer. The downstream proteins, including PERs, CRYs, DBP, RORs and REB-ERBs. RORs and REB-ERBs reversely regulate the transcription of BMAL1, as well as the E4BP4 form the secondary loop. The E4BP4 and DBP form as the controller of the third loop.

It has been reported that disruption of CR homeostasis results in multiple diseases, including cardiovascular diseases, obesity, type II diabetes, and cancer [70]. Studies indicated that disruption of CR contributes to the hallmark of cancer, including the cell cycle, DNA damage repair, apoptosis, and cell metabolism [70]. However, the link between the TME and CR disruption in promoting tumor growth and metastasis is not clear. A study used CAFs and healthy fibroblasts co-culture with a colorectal cancer cell line HCT116, finding cancer cells with healthy fibroblasts have milder proliferation than those with CAFs. They found various growth factors and cytokines from CAFs paracrine affected the tumor cell proliferation and CR genes transcription [75]. Another study with CAFs in the pre-clinical mouse model showed CR disruption activated the stromal niche in tumors. These tumors demonstrated high ECM and cytokine production with a WNT10 protein expression in the stroma. Tumor tissue with high WNT10 expression contained metastasis-promoting properties [76]. Some other studies indicated the tumor host CR was affected by the tumor cell [69]. For example, a study used *Kras* gene mutated mice with lung adenocarcinoma model showed the tumor cells distally rewired the CR transcriptome and metabolome in the host liver [77]. Tumor cell secreted IL-6 to alter liver CR and dampen insulin and glucose sensitivity [77]. Another study found the host CR was tightly regulating the macrophages responses, including cytokine and chemokines secretion, phagocytic ability, and monocyte recruitment from bone marrow [78]. The host macrophage response deficiency was associated with the aging cells, which were absent of CR gene transcription. A tumor suppress gene, Kruppel-like factor 4 (Klf4) that regulate cell differentiation and reprogramming, showed low expression in aged macrophages. The study showed losing of Klf4 expression was associated with the CR disruption in innate immune homeostasis [78]. They also found the expression of Klf4 was associated with Bmal1, but Bmal1 expression was not related to age. They assumed other transcription factors were involved in this correlation.

Although the link between CR and cancer is established, it remains challenging to adapt therapeutic approaches. So far, the well-established pharmacological approaches are using melatonin and orexin. At night, the SCN induced noradrenaline (NE) to stimulate melatonin synthesis in the pineal gland. Melatonin binds to its receptors 1 and 2 to suppress neuronal firing and facilitate sleep and exert the phase-shifting effect on CR [79]. The orexin is stimulated by SCN in the daytime to maintain wakefulness. Neurons release orexin A and B, and they bind to orexin receptor 1 and 2, to serve the function as suppression of rapid eye movement (REM) sleep [79]. Melatonin or orexin monotherapy and combination therapy have been used in patients with insomnia and jet lag. However, there are very limited studies on using melatonin or orexin in treating tumor host with CR disruption. Some studies used small chemicals to target the core CR genes expression in cells and at mouse level, showing well anti-tumor effect. For example, the inhibitor of CRY protein could activate E-box transcription and reduce

amplitude, had been used in inhibiting breast cancer cell [79]. However, further extension of the preclinical pharmacological studies into clinical trials with CR regulation treatment is necessary. Apart from pharmacological approaches, studies indicated lifestyle education, healthy diet improvement and environmental alteration could also become supplementary approaches in CR disruption therapy.

3 RESEARCH AIMS

1. To resolve AAD resistance in cancer therapy and provide a new therapeutic paradigm by targeting stromal components.
   Paper I: To reveal AAD resistance in tumors with adipocytes or lipid-rich environment through FFAs uptake. Inhibiting the key enzyme of the β-oxidation pathway reduced FFA uptake and reduced tumor growth.
   Paper II: To enlarge the BAT into megaBAT through targeting PDGFRα signaling in preadipocytes. MegaBAT with functional adipocytes will become a novel therapeutic paradigm for cancer and metabolic diseases.
   Paper III: To resolve AAD resistance in high FGF-2 expression tumor by combination therapy, which targeting on VEGF and PDGFRβ.

2. To reveal the role of stromal cells, especially CAFs and TAMs in cancer growth and metastasis.
   Paper IV: To understand the molecular mechanisms of the interaction between CAFs and TAMs in PDAC and provide a novel therapeutic strategy for treating PDAC.
   Paper V: To reveal the molecular mechanism of CR disruption and tumor growth and metastasis through the impact of CAFs.

4 MATERIALS AND METHODS

All experimental details were listed in constitute papers. I have listed some special experiments below.

4.1 ADIPOCYTE DIFFERENTIATION ASSAY

Mouse BAT1 cells were maintained in Dulbecco's modified Eagle medium (DMEM)/F-12 supplemented medium (2437064, Gibco) with 10% fetal bovine serum (FBS) (Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin (P4333, Sigma-Aldrich). 24-well plates were coated with gelatin for 1 h. Approximately, 2×10^4 cells were seeded in each well. Differentiation of progenitor cells was induced by addition of 0.5 nM 3-Isobutyl-1-methylxanthine (IBMX), 1 μM dexamethasone, 0.5 μg/mL insulin, 1 μM rosiglitazone, and 1 nM triiodothyronine for 4 days followed by treatment with 0.5 μg/mL insulin and 1 nM triiodothyronine for another 4 days.

4.2 DUAL LUCIFERASE REPORTER ASSAY

A psiCHECKTM-2 vector and a Dual-Luciferase® Reporter Assay System (C8021, E1910, Promega) were used for target validation. Two approximately 700 bp sequences of the 3'UTR of Pdgfra mRNA that contained either miR-485 binding site 1 (bs1) or bs2 were separately cloned into a psiCHECKTM-2 vector. HEK293T cells (ATCC) were transfected with a transfection medium containing recombinant plasmids (1 μg/μL DNA) mixed with a Polybrene® medium for 16 h. Cells were further transfected with the miR-485 mimic using the DharmalFECT®siRNA transfection reagent. Renilla and firefly luciferase signals were measured at 48 h after the initial transfection using the Dual-Luciferase® Reporter Assay System and a Sirus L Tube Luminometer (Titertek-Berthold). To mutate the binding sites of miR-485 in the 3'UTR of Pdgfra mRNA, PCR amplification with primers containing 5 mismatches in the miR-485 bs1 or bs2 were used and the mutated recombinant plasmids were inserted into the psiCHECKTM-2 vector the further luciferase quantification.

4.3 MOUSE MODELS AND TREATMENT

All animal studies were approved by the Northern Stockholm Ethical Committee for animal experiments in Sweden (N7/16, 6196-2019, N3/17+N193/13, N2/17+N192/13), the Fudan University Ethical Committee and the Ethical Committee of Tokushima University for Animal Research. C57BL/6 wild-type mice were purchased from the Janvier Laboratory, Germany. C57BL/6 mice at the ages of 4 to 5-month-old were fed with high fat diet (HFD) or normal diet were used and maintained in animal facilities of Karolinska Institutet. NOD. Cg-*Prkdc*^{scid} *Il2rgt*^{m1Wjl}/SzJ (NSG) mice and CB17/lcr-Prkdc^{scid}/lcrCrl (SCID) were from the Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden. MiR-485 KO mice were generated by Dr. Akihiro Yasue at Tokushima University, Japan. C57BL/6-T1/St2$^{-/-}$ were provided by Padraic Fallon at the Trinity College Dublin, Ireland. Bmal1$^{+/-}$ mice as breeding founders were kindly provided by Dr. Garret A. FitzGerald at the Institute for

Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, USA. Bmal1$^{-/-}$ and Bmal1$^{+/+}$ mice were confirmed by genotyping of ear tissue. Genotyping was performed by a PCR-based method. Primers were listed in Table 1. All mice were housed in animal facilities with constant temperature (20 ± 2 °C), humidity (50 ± 10%), and a 12 h-light and 12 h-dark exposure cycle each day.

For the subcutaneous tumor model, approximately 3 × 10^6 tumor cells in 100 μL PBS were implanted subcutaneously into the dorsal region of each mouse. Tumor volumes were measured with a caliper and calculated according to the standard formula (length × width^2 ×0.52). The endpoint of tumor volume is 2.0 cm^3. Mice were euthanized by inhalation of overdose of isoflurane (Attane, VM Pharma) and tumor tissues were dissected. For the orthotopic tumor model, approximately 1 × 10^6 cells in 25 μL PBS were injected to liver and spleen. Three weeks after tumor implantation, mice were sacrificed, and liver and spleen were dissected.

Pharmacological and antibody treatments were delivered by intraperitoneal injection, intravenous injection, gavage, or local tissue injection to each mouse.

4.4 INDIRECT CALORIMETRY OF MICE

Metabolic rates were quantified by measuring oxygen (O_2) consumption and carbon dioxide (CO_2) production of each mouse over a defined time using the module-based calorimetry systems (Ul-2, Sable Systems International of CLAMS). An oxygen sensor was heated for a least 6 h prior to calibration with the reference gases–100% nitrogen gas and a mixture of 20.5% O_2 and 0.5% CO_2. For measuring the basal level of metabolism, mice were transferred to an Oxymax chamber equipped with different temperatures. The area under curve ^{50} of the volume of O_2 (VO2) consumed or CO_2 (VCO2) was calculated for 30 min. Three to five mice from each group were measured.

4.5 CELLULAR COMPONENTS ISOLATION FROM TUMOR

Fresh tumor tissue was cut into small pieces and incubated in a mixture of 0.15% type I collagenase (C0130-500MG, Sigma-Aldrich) and type II collagenases (C6885-1G, Sigma-Aldrich) in PBS at 37 ºC for 1h. The single cell suspension was filtered with a 100 μm cell strainer and followed by a 70 μm cell strainer. Cells were stained with surface protein markers for 45 minutes on ice, followed by an Alexa Fluor 647-conjugated donkey anti-rat secondary antibody. A rat anti-mouse FAP (MAB9727, R&D systems) antibody was used for fibroblasts. A rat anti-mouse F4/80 (123122, BioLegend) antibody was used for macrophages. A rat anti-mouse CD31 (553370, BD Biosciences) antibody was used for endothelium cells. The stained samples were filtered with a 40 μm cell strainer, followed by fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS). In FACS sorting, stained cells were sorted by FACS Aria III (BD Bioscience) using a BD FACSDica software. In MACS, anti-Alexa Fluor 647 MicroBeads (130-091-395, Miltenyi Biotec) were subsequently used for magnetic labeling. After washing, cells were sorted with a MACS column and magnetic MACS separators (Miltenyi Biotec). The single cell suspension stained with secondary antibody as a

negative control. After sorting, single-cell suspension was collected for further culture or detection.

4.6 MICROARRAY AND RNA SEQUENCING

Total RNA and microRNA were isolated from adipose tissue SVF, tumor SVF or F4/80^{+} and negative cells. A genome wide Affymetrix mouse gene 2.0 ST microarray. Normalization and analysis for differentially expressed genes were performed using robust multi-array analysis and significance analysis of microarrays [8] via R statistical software packages "oligo" and "samr". Heatmaps were presented for significantly up- and down-regulated genes using the Microsoft Excel 2021. MiRBase was used for obtaining miRNA sequences and micro RNA.org, PicTar, TargetScan, and miRDB were used for the identification of potential miRNA targets.

4.7 ETHICAL CONSIDERATION

All mouse experiments in this thesis were approved by the Northern Stockholm Ethical Committee for animal experiments in Sweden and the Ethical Committee for animal experiments of the Fudan University in China. The human sample experiment was approved by the Ethical Committee of Shuguang Hospital Affiliated with Shanghai University of Traditional Chinese Medicine, China.

The experimental mice were housed in animal facilities with the individually ventilated cage (IVC) maximum of five mice were kept in one cage. Constant temperature (20 ± 2 °C), humidity (50 ± 10%), and a 12h-light and 12h-dark exposure cycle each day were provided in animal facilities. The food for mice, including normal diet and high-fat diet (HFD) were given freely. When conducting surgical procedures and tumor cell implantation, mice were under isoflurane (Attane vet, QN01AB06, VM Pharma) inhalation. Painkiller was delivered to mice in constant three days after surgical procedures. All animal experiments followed the ethical approvals and the 3Rs (replacement, reduction, and refinement) framework.

Our collaborators conducted the human PDAC sample experiment in the Shuguang Hospital Affiliated with Shanghai University of Traditional Chinese Medicine, China. All experiments followed the ethical approval.

5 RESULTS

5.1 AAD RESISTANCE IN CANCER WITH ADIPOCYTES

Although targeting tumor blood vessels is a common strategy in cancer therapy, tumor grown with adipocytes or lipid-rich environment, for example, breast cancer, PDAC, and CRC, showed resistant to AAD therapy [36,80-82]. To resolve this resistance mechanism and improve drug efficacy, in project I, we developed a mouse resistant model of AAD therapy by implanting CRC and PDAC tumor cells to inguinal white adipose tissue (iWAT), as well as an orthotopic model of HCC to steatotic liver. With the subcutaneous and orthotopic mouse tumor model, we could see that the tumor grown in iWAT and steatotic livers were resistant to AAD therapy. In contrast, tumors grown in subcutaneous tissues and healthy liver demonstrated sensitivity to AAD therapy. Under histological analysis, those tumors grown adjacent to AT demonstrated markedly tumor vessels reduction similarly to the tumors in non-adipose tissues and further elevated hypoxia in the TME. Hypoxia is one of the major factors in triggering tumor growth and invasion [49,83]. Tumor cells under hypoxia increase glucose uptake and glycolysis, which is known as the Warburg effect [84,85]. However, the reduction of tumor vessels would restrict glucose uptake in AAD-treated tumors. In this case, we assumed tumor cells used another resource as energy support.

We performed a genome-wide expression profiling in the CRC-WAT tumor tissues, which demonstrated the expression of fatty acid oxidation (FAO) related genes, FFA transporter-related genes and energy metabolism-related genes had markedly increased after AAD treatment. This result suggested tumor grown in adipose tissue obtained energy through lipid metabolism. To corroborate these findings, we examined the FAO related regulator and AMP-activated protein kinases (AMPK) in the CRC and HCC tumors treated with AAD. A high level of AMPK and its phosphorylated protein (p-AMPK) were detected, indicating that AAD-triggered hypoxia promoted FAO pathway activation. To further confirm the AAD induced hypoxia had triggered AMPK activation, we detected *in vitro* cancer cells under hypoxia. Finding AMPK activation in these cells. In addition, the FFA had stimulated tumor cell proliferation with low glucose levels under hypoxia condition. The expressions FFA transporter-related genes had also elevated. We also found the expression level of multiple FFA transporters had markedly increased in AAD-treated steatotic liver CRCs compared with the control tumors, including *Cpt1a*, *Cd36*, *Fapb1*, *Fabp4*, *Slc27a2* and *Slc27a5*. With the inhibition of FAO limiting enzyme carnitine palmitoyltransferase 1 (CPT1), the effect of FFA in the hypoxia tumor had markedly decreased. CPT1 is the enzyme transfer FFA into mitochondria for FAO. These data demonstrated that hypoxia triggered proliferation and FFA uptakes in tumor cells with a lipid surrounding environment.

To further confirm the impact of the FAO-related metabolic reprogramming on tumors in steatotic livers, we used a small hairpin RNA (shRNA) to knockdown the *Cpt1* gene in tumor cells. After knocking down *Cpt1*, the cells were implanted to mice with steatotic livers. Both CRC and HCC tumor with *Cpt1* knockdown had showed sensitive to anti-VEGF treatment.

We further used a combination treatment of CPT1 inhibitor and anti-VEGF in a CRC tumor with steatotic livers. With the pharmacological inhibition of CPT1, CRC in steatotic livers showed sensitive to anti-VEGF treatment. The size of tumors markedly decreased with combination therapy.

Our data revealed the AAD therapy resistance in tumors grown with CAAs or lipid-rich environment. Tumor vessels were markedly reduced with AAD treatment, however, AAD treatment-induced hypoxia resulted in lipid lipolysis and FFA released. FFA serves as another energy resource to support tumor growth. After the inhibition of FAO limiting enzyme CPT1, the resistance of AAD had reversed.

5.2 ENLARGED BROWN ADIPOSE TISSUE SIZE BY TARGETING PDGFRA SIGNALING IN VIVO

Targeting the cancer metabolic reprogramming provided us a novel idea in cancer therapy. Recently, our group published a paper about cold-induced BAT activation suppressed tumor growth by increasing the non-shivering thermogenesis (NST) [86]. Under cold exposure, the activated BAT consumed blood glucose in tumor host, which deprived glucose support of tumors and downregulated the transcription of glucose transporter genes in tumors. BAT activation suppressed tumor growth by altering metabolism in tumor host. This finding suggested the BAT as a novel therapeutic tool for cancer therapy. To further activate the BAT for cancer therapy, we invented a genetic method to enlarge the mass size of the BAT *in vivo*.

In human infants, BAT represents approximately 5% of the body mass [87] but with aging, the mass of BAT becomes tiny [88]. In our project II, we found an approach to downregulate the BAT progenitor cell marker PDGFRα signaling *in vivo*, which triggered more progenitor cell differentiation and enlarged the mass of BAT. To begin with, we used a primary BAT progenitor cell line, BAT1 cells, to study the function of PDGFRα. We found that along with cell differentiation, BAT1 cells accumulated lipid droplets and turned into mature brown adipocytes and gradually lost PDGFRα expression. We then used pharmacological and genetic method to inhibit the PDGFRα in BAT1 cells, finding more cells differentiated into mature brown adipocytes and with high expression of browning markers, compared to non-treated cells.

To study the impact of PDGFRα in the progenitor cells of BAT, we used an anti-PDGFRα neutralizing antibody to treat mice *in vivo* and put them in both thermoneutrality and cold environment, finding that BAT mass size increased into a mega size (megaBAT) and cell number was increased compared to NIIgG treated group under thermoneutrality and cold. The expression level of progenitor cell markers, including myogenic factor 5 (MYF5), stem cell antigen 1 (Sca-1) and PDGFRα, were diminished under PDGFRα blockade, indicating there were more progenitor cells had differentiated into mature brown adipocytes. A genomic-wide profiling was used to detect the key molecular regulators of the BAT stromal vascular fractions (SVFs) from CL 316,243 (CL)-treated WAT. The CL as a β3-adrenoreceptor agonist can mimic the cold exposure effect in WAT and BAT. In the genomic-wide profiling of both

microRNA and mRNA, we found the microRNA-485 (miR-485) was the unique miRNA targeting PDGFRα.

To test the pathophysiological functions of miR-485, we treated normal diet mice and high fat diet (HFD) mice with an adenoviral miR-485 (Adv-miR-485) in BAT local injections and put them into thermoneutrality and cold environment. Similar to the treatment with anti-PDGFRα neutralize antibody, the BAT had become megaBAT with miR-485 treatment, and total number of cells in megaBAT was increased, compared to the vehicle control in both thermoneutrality and cold. The BAT activation markers, including uncoupling protein 1 (UCP1) and cytochrome c oxidase subunit 4 (COX4) had markedly increased in megaBAT under cold exposure. We further detected the global metabolism of HFD mice with megaBAT. Under cold exposure, activated megaBAT markedly improved global metabolism by increasing the NST [89] in HFD mice. They showed lower glucose level and improved glucose tolerance test (GTT), as well as higher sensitivity to insulin by decreasing insulin level and improved insulin tolerance [90]. Meanwhile, the blood lipid profiling, including cholesterol, FFA, triglyceride and glycerol, was significantly decreased in HFD mice with megaBAT. Liver histological analysis indicated the liver steatosis in HFD mice was markedly improved by Adv-miR-485 treatment. Loss-function experiment using CRISPR-Cas9 to generate a miR-485 knock-out (KO) mouse model. These miR-485 KO mice with a HFD feeding showed a higher expression of PRDGFRα and larger individual adipocyte in the BAT compared to the wild-type control mice. In this model, there was a lower activation of UCP1 and COX4 in the BAT. The miR-485 KO mice also had higher blood glucose level than the wild-type controls.

In this project, our data provided a novel tissue manipulating method *in vivo* to enlarge BAT mass and further improve the whole-body metabolism in HFD mice. Pharmacological and genetic experiments proved PDGFRα is a gatekeeper in BAT differentiation. Inhibition of the PDGFRα pathway triggered BAT progenitor cell differentiation and the BAT turned into megaBAT.

5.3 RESOLVE AAD RESISTANCE BY COMBINATION THERAPY

Another mechanism of AAD therapy resistance is related to compensatory angiogenic factors apart from VEGF. It is known that FGFs and PDGFs play crucial roles in angiogenesis [30,37]. While VEGF contributes mainly to vascular endothelial cell migration and tips formation. The FGF-2, one isoform of the FGF family, serves as a trigger to stimulate the proliferation of ECs by binding to its receptor on ECs [29,30]. FGF-2 also plays a role in pericytes recruitment to and proliferation through binding to its receptor and interact with PDGF-B-PDGFRβ signaling pathway [30,91]. PDGFs target perivascular cells, including the pericytes and vascular smooth muscle cells, and contribute to remodel the vascular structures through the PDGF-B-PDGFRβ signaling pathway [91]. Conventional AAD therapy mainly targets VEGF and its receptors on endothelial cells, particularly VEGFR2. Our data in project III provided a new paradigm of FGF-2 off-target AAD therapy, by combination therapy of inhibitors of VEGF and PDGFs could overcome the resistance effect of AAD.

To investigate the effect of FGF-2 on tumor tissues, we overexpressed FGF-2 in breast tumor and fibrosarcoma cell lines. After implanted to mice, we found that tumors with high FGF-2 expression showed a high growth rate with significant proliferation and low apoptosis. It was confirmed by a Ki67 and pimonidazole staining in tumor tissues. Under the histological and immunofluorescence analysis, we found the high FGF-2 tumors had markedly elevated vessel density, pericytes coverage and numbers. Under anti-VEGF monotherapy, high FGF-2 tumors demonstrated no response while low FGF-2 tumor showed sensitive to the treatment. The monotherapy of anti-PDGFR drug imatinib demonstrated no effect on inhibiting both high FGF-2 and low FGF-2 tumor, but the pericyte coverage rate was markedly decreased due to a PDGF-dependent pericyte recruitment mechanism. Because accumulated pericytes served as a shield of tumor vessels, monotherapy with anti-VEGF could not directly target on endothelial cells, which led to resistance to anti-VEGF therapy. With this data, we invented a novel strategy using a combination therapy of anti-VEGF and imatinib in high FGF-2 tumors. Surprisingly, high FGF-2 tumors showed marked response to combination therapy. Under immunofluorescence analysis, we found that high FGF-2 tumors with combination therapy demonstrated fewer vessels and a lower endothelial cell proliferation rate and higher apoptosis. Meanwhile, blood vessels demonstrated lower blood perfusion and vessel leakiness, observed by lysinated Rhodamine-labeled 2000 kDa and 70 kDa dextrans.

Since imatinib is a drug targeting both PDGFRα and PDGFRβ, to further prove the specific signaling pathway, we used a PDGFRβ neutralize antibody combined with anti-VEGF to check the anti-tumor effect. Consistent with our findings using imatinib, the dual antibody combination showed significant anti-tumor efficacy on high FGF-2 tumors. With dual antibody treatment, high FGF-2 tumors showed lower vessel density and blood perfusion.

In this project, we investigated the mechanism of AAD resistance through other angiogenic factors. Combination therapy on FGF-2 off-target tumors with dual inhibition of VEGF and PDGFRβ could overcome this resistance.

5.4 TARGETING CAF AND TAM SIGNALING IN TUMOR

Apart from the vascular structure and adipocytes, CAFs and TAMs play important roles in the TME. In project IV, we tried to explain the cell-to-cell communication between CAFs and TAMs, as well as their functions in terms of metastasis. Study found that the TME with hyperfibrotic structures relates to a poor tumor prognosis and survival [92]. In some types of tumors, for example, PDAC, there can be 90% of tumor mass consisted of stromal components [93], including fibroblasts and myofibroblasts, and ECM [7]. Studies indicated a high ratio of ECM and fibrotic structures in tumor tissue could increase the tumor metastasis [94]. In our project, we confirmed the human PDAC samples had high fibrotic and inflammatory markers expression. We found the IL-33 was the most upregulated cytokine in PDAC samples compared to adjacent healthy pancreatic tissue. The IL-33 was mainly produced by pericytes and inflammatory CAFs (iCAFs). Consistent with high expression of IL-33, its receptor, ST2, was markedly increased in TAMs, which was marked by $F4/80^{+}$ and $CD163^{+}$ staining in mouse and human PDAC

samples, respectively. These findings suggested a high IL-33 expression was associated with TAMs infiltration in PDAC.

To investigate the molecular signaling in IL-33 mediated TAM, we stimulated the macrophages with IL-33 *in vitro* and conducted genomic-wide expression profiling. We found the *Cxcl3* gene was the most upregulated gene after stimulation. To further prove the IL-33-ST2-CXCL3 signaling pathway, we utilized a ST2 knockout (KO) mice model with PDAC. Ablation of ST2 significantly decreased CXCL3 expression. This result was also confirmed by *in vitro* experiment. By analyzing the human PDAC TCGA data, we found the IL-33 and CXCL3 had a matched correlation expression pattern. Taken together, these results demonstrated CXCL3 expression level was positively correlating with IL-33/ST2 signaling.

To further investigate the signaling pathway of IL-33 stimulating CXCL3 expression in macrophages, we analyzed the downstream signaling of IL-33/ST2. We found that mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK) and p38 were activated. Inhibition the ERK and p38 significantly ablated IL-33-induced CXCL3 expression on the F4/80$^+$ population of PDAC. This result indicated the F4/80$^+$ monocytes/macrophages were the primarily responsible for CXCL3 expression, which via the ERK/p38-mediated regulation. In order to investigate the transcriptional regulation, we used a transcriptome study to define transcription factors involved in IL-33-stimulated macrophages, we found the *Myc* gene was significantly upregulated. We then used the siRNA and a pharmacological approach to inhibit the *Myc* gene, the IL-33-induced CXCL3 expression was significantly blocked in both monocyte cell line and F4/80 isolated TAMs. These results confirmed the IL-33-ST2-ERK/p38-MYC-CXCL3 signaling pathway was the main signaling in macrophages of PDAC.

Next, we explored the CXCL3 expressing cell types in PDAC. We detected the CXCL3 and its receptor, CXCR2, in PDAC tissues by immunostaining. It demonstrated the CXCL3 proteins were mainly expressed on TAMs, and the CXCR2 proteins were mostly expressed on α-smooth muscle actin (α-SMA$^+$) myoCAFs. This result had been confirmed by various mouse PDCA tumors and human patient samples. The monocytes/macrophages showed a moderate CXCR2 expression, and no expression was detected in cancer cells. We applied pharmacological inhibition and genetic deletion of ST2 in mouse PDAC model, finding a markedly decreased of CXCR2 and PDGFRβ expression in PDAC tumors. By analyzing the human PDAC and TCGA data, we validated the high CXCR2 expression in tumor. Other fibrotic markers, including PDGFRB, fibroblast activation protein (FAP) and Podoplanin (PDPN) expression were correlated with CXCR2 expression. These data supported the CXCR2 is mainly expressed in PDAC-CAFs.

To further confirm the biological function of CXCL3 and CXCR2 in CAFs, we applied a genomic profiling to analyze gene signature upon CXCL3 stimulated fibroblasts. The gene of α-SMA was identified as one of the most upregulated genes. qPCR analysis further revealed α-SMA and other fibrotic markers, including vimentin and desmin, were significantly increased upon CXCL3 stimulation. These results indicated CXCL3 induced a fibroblast to myofibroblast transition. Furthermore, the inhibition of CXCR2 by siRNA had markedly

decreased the fibroblast to myofibroblast transition under the stimulation of CXCL3. With the inhibition of ST2 by a soluble ST2 antibody or ST2 knockout strain in the PDAC tumors, we found the expression of α-SMA was significantly decreased. We further validated these findings by using the human genome database. Compared to the healthy pancreatic tissues, PDAC samples showed a higher *ACTA2* mRNA expression. Notably, high expression of *ACTA2* correlated with poor disease-free survival (DFS).

With this result, we assumed that CXCL3-primed myofibroblasts were the key players in tumor metastasis. To validate the assumption, we applied an *in vitro* coculture of CXCL3-treated myofibroblasts with cancer cells. We found that fibroblasts and cancer cells formed clusters and CXCL3 further triggered cluster formation. Gene profiling analysis showed that collagen III was the most upregulated cell adhesion-related gene in the CXCL3-stimulated fibroblasts. Inhibition of the collagen III gene had markedly decreased CXCL3-stimulated fibroblast motility. To confirm that TAMs-CAFs signaling transition promotes PDAC metastasis, we established a mouse model by depleting TAMs. After TAMs pharmacological depletion, metastasis had markedly decreased in PDAC.

With all these data above, we revealed the mechanism of the CAFs-TAMs transition by IL-33-ST2-CXCL3-CXCR2 pathway. CXCL3 primed CAFs proliferation and morphological transformation to myofibroblasts. The myofibroblasts produced collagen III in ECM and formed clusters with cancer cells. Eventually, myofibroblasts with high motility metastasis with cancer cells. This new mechanism provided a therapeutic idea about targeting TAMs and CAFs in PDAC might prevent PDAC metastasis.

5.5 TARGETING CAF IN CANCER HOST WITH CIRCADIAN RHYTHM DISRUPTION

Circadian rhythm (CR) is the endogenous regulator in the mammal body that oscillates with the natural light on earth to maintain body's physiological process and homeostasis. Researchers found CR disruption would cause various diseases, for example, cardiovascular diseases, obesity, metabolic diseases and even cancer [69,70,79]. Our project V is to reveal the correlation between CR disruption and cancer progression. *Bmal1* gene is one of the key genes to maintain CR system in mammals. In our study, we used a *Bmal1* gene knockout (KO) mouse model with CRC, PDAC and HCC tumors to reveal the TME alteration in the CR disruption host. *Bmal1* wild type (wt) mouse with tumors was used as a control. Surprisingly, we found tumors in the *Bmal1* KO mice showed accelerated tumor growth rate. Histological analysis demonstrated hyperfibrotic structures in the TME. Immunofluorescent analysis with fibroblast markers, including FAP, α-SMA, DESMIN, PDGFRα and PDGFRβ had markedly higher expression in the *Bmal1* KO mice, which indicating the tumor of KO mice had more fibroblasts. With this result, we assumed that CAFs in the *Bmal1*KO mice may accelerate tumor growth.

To further investigate the expanded fibroblast function in the CR disruption host tumors, FACS-isolated SVFs from the CRC tumors were subjected to genomic-wide expression analysis. This profiling analysis demonstrated differentially expressed genes were specifically

involved in cell migration, fibroblast migration and ECM formation pathways. In these pathways, the *Serpine1* gene was the most downregulated gene in the tumor SVF of the *Bmal1* KO mice. Serpine1 encodes the protein plasminogen activator inhibitor 1 (PAI-1). *Serpine1* gene expression was further confirmed by qPCR in CRC tumors, tumor SVF, and isolated CAFs. With the transcription study, it was reported that *Bmal1* directly controlled the transcription of *Serpine1* gene. The BMAL1 and CLOCK proteins form as a heterodimer to target on the E-box of the Serpine1 [73,95,96]. Therefore, the *Bmal1* KO host had limited heterodimer to trigger Serpine1 transcription, resulting in lower Serpine1 protein expression. We then analyzed the protein level of PAI-1 and its downstream signaling proteins, including tissue plasmin activator (tPA) and urokinase (uPA) in CRC tumors, tumor SVF and CAFs. We found the PAI-1 expression was negatively correlated with *Bmal1*. In contrast, the expression of tPA and uPA were both robustly increased with a diminished expression of PAI-1. Apart from immunoblot experiments, we also tested enzyme activity levels of tPA and uPA, which were consistent with our immunoblot results.

Accumulated tPA and uPA in tumor tissues continue to activate the plasminogen in plasma, which converted plasminogen into plasmin. We confirmed the elevated plasmin level in CRC tumors and tumor-bearing mouse plasma. Active plasmin could contribute to many reactions in tissue; for example, it could convert latent TGF-β into active TGF-β. To validate this point, we detected active TGF-β level in both CRC tumors and tumor-bearing mouse plasma. The active TGF-β was markedly increased in the tumors of the *Bmal1* KO mice. We also detect the phosphorylation of Smad2 (pSmad2), which was one of the key downstream products of activated TGF-β. The phosphorylated Smad2 was markedly increased in the tumors of *Bmal*1KO mice. In order to investigate the biological function of TGF-β in CAFs, we cultured the isolated CAFs from the *Bmal1*KO and wt mice. After a 48h of cultivation, the CAFs from the *Bmal1*KO mice showed an accelerated proliferation rate and high motility manner. Taken together, these findings indicated the *Bmal1* deletion promoted CAFs expansion through a PAI-1-tPA/uPA-plasmin-TGF-β axis.

Next, we applied a TGF-β blockade in wt and the *Bmal1* KO tumor-bearing mice. The treatment with an inhibitor of TGF-β receptor I showed a marked reduction of tumor growth rate in the *Bmal 1* KO mice but not wt mice. Immunofluorescence staining showed various fibrotic marker expressions diminished in the tumors of the *Bmal 1* KO mice with TGF-β blockade. The isolated CAFs with a TGF-β inhibitor treatment demonstrated a lower proliferation rate and lower motility. These results confirmed the CAFs contributes to tumor growth in the *Bmal1* KO mice through the PAI-1-tPA/uPA-plasmin-TGF-β signaling axis.

We further explored the effect of CR disruption on tumor metastasis by a spontaneous metastasis model. In the *Bmal1* KO mice, we found approximately 83% of tumor-bearing had visible metastases in the lung and liver. In contrast, only 33% of visible metastasis were observed in control wt mice. To detect the function of *Bmal1* KO CAFs in metastasis, we co-implanted tumor cells with wt or *Bmal1* KO CAFs to C57BL/6 mice. Consistent with the transgenic mice result, tumors with *Bmal1* KO CAFs showed markedly promoted metastasis.

Under immunofluorescence analysis, we found the metastatic lesions in the *Bmal1* KO group demonstrated higher expression of fibroblasts markers, which indicates *Bmal1* KO CAFs had a fibrotic phenotype and promoted cancer metastasis. After TGF-β inhibition, the ratio of metastasis decreased significantly. With these data, we concluded that Bmal1-PAI-1-tPA/uPA-plasmin-TGF-β axis was involved in tumor growth and metastasis of CR disrupted host. Potentially targeting this axis and preserving CR system homeostasis may become a novel therapeutic strategy in cancer patients.

6 DISCUSSION

6.1 SUMMARY OF THE MAIN FINDINGS

In this thesis, we provided evidence to show the stromal cellular components play important roles in cancer growth and metastasis. We further presented novel ideas of targeting the stromal cells for cancer therapy. In paper I and III, we investigated the resistance mechanism of AAD therapy in cancer with lipid-rich environment and high FGF-2 expression as an alternative pro-angiogenic factor. By inhibiting FFA transporters as well as using dual antibodies to inhibit VEGF and PDGFRβ, the resistant effect was reversed. In paper II, we investigated a method by inducing BAT progenitor cell differentiation, leading to form a mega-size BAT mass in adults. The megaBAT can become a therapeutic approach in cancer. In paper IV and V, we focused on the function of the CAFs in cancer. We found that CAFs and TAMs cross talked through the IL-33-ST2-CXCL3-CXCR2 axis. TAMs triggered CAFs proliferation and transition into myofibroblasts. Myofibroblasts formed clusters with cancer cells and supported cancer metastasis. We also revealed the link between circadian rhythm (CR) disruption and cancer metastasis through the effect of CAFs. The CR core gene *Bmal1* deletion resulted in CAF's proliferation, which through the PAI-1-tPA/uPA-plasmin-TGF-β axis. Active TGF-β transited CAFs into myofibroblasts, and myofibroblasts promoted cancer metastasis.

6.2 INTERPRETATION OF UNDERLYING MECHANISMS

In this thesis, there are two main topics. The first one is to resolve AAD resistance in cancer therapy, the other is to reveal the roles of stromal cells, especially CAFs and TAMs, in promoting cancer growth and metastasis.

6.2.1 Resolve AAD resistance in cancer therapy and provide a new therapeutic paradigm by targeting stromal components (Paper I, II and III).

Regarding the AAD resistance, we mainly used anti-VEGF-based therapy. Because VEGF is the most predominant angiogenic factor, which promotes angiogenesis, vascular permeability, and vascular remodeling [38,97]. So far, there are 14 AADs approved by the US Foods and Drug Administration (FDA) in the clinic [89]. Most of them are monoclonal antibodies that specifically target to VEGF, resulting in the protein being unable to bind to its receptors. Other types of drugs bind to other angiogenic factor receptors on the surface of ECs, as well as the proteins in the downstream signaling pathways [97].

In order to resolve the resistance of anti-VEGF therapy, we should further understand the mechanisms of resistance. Some researcher divided cancer drug resistance into intrinsic and acquired resistance. Intrinsic resistance refers to cancer having no response from the beginning of the therapy, and acquired resistance refers to cancer show responses initially but no progression or regression along with time [98]. According to some studies, the mechanisms of AAD resistance could be classified into these aspects: 1) intrinsic effects of cancer cells lining with ECs or ECs derived by cancer stem cells; 2) intrinsic effect of pericyte protecting ECs; 3)

acquired resistance by other angiogenic factors compensation, for example, PDGF, FGF, as well as hypoxia over triggered VEGF expression; 4) acquired effect of bone marrow-derived cells (BMDC) turned into ECs [38]. In Paper III, the resistance of anti-VEGF-based AAD confirms the mechanism of intrinsic effect since cancer cells produced high FGF-2 protein bound to its receptor and activated the PDGFRβ by producing PDGF-BB and PDGF-DD. Pericytes expressed high PDGFRβ were recruited to the ECs and formed a shield against drugs.

In our Paper I, data demonstrated another resistance mechanism: anti-VEGF-based AAD induced metabolic reprogramming of cancer cell from glucose dependence into FFA dependence. The anti-vessels effect was promising according to our data. However, because of the metabolic reprogramming, the tumor suppression effect of AAD was limited. This novel finding indicated an anti-tumor effect resistance instead of an anti-VEGF resistance mechanism, which suggested the tumor development and survival is tightly depending on the metabolism of TME. To tackle this dilemma, we need to consider inhibiting the cancer metabolic reprogramming and targeting the intrinsic mutation of cancer cells.

In this study, hypoxia is the troublemaker to induce metabolic reprogramming from glycolysis to lipolysis, which resulted from the adjacent adipocytes to release FFA. Hypoxia also upregulated the transporters of FFA on cancer cells, triggered the fatty acid oxidation (FAO) pathway. Under hypoxia, the accumulation of hypoxia-inducible factor 1 (HIF1α) reversely induced VEGF expression. Therefore, using hypoxia inhibitors may prevent this reprogramming and VEGF expression. So far, there are various therapeutic approaches to inhibit hypoxia, which are specifically targeting the hypoxia-inducible factor 1 (HIF1). However, since the contrary effects of HIF1 in acute tissue effect and chronic diseases, the inhibitors of HIF1 in cancer are still in pre-clinic level [99,100]. Also, according to our results in paper I, the hypoxia-induced lipolysis and FAO pathway may deserve further investigation. According to the classical Warburg effect, the cancer cells produce ATP through anaerobic respiration even with the presence of oxygen, which is a step one metabolic reprogramming [62,84]. With the treatment of AAD and cancer vessels regression, the presence of oxygen constantly decreased, cancer cells switched to uptake FFA and produced ATP through FAO pathway, which is forming a step two metabolic reprogramming. Therefore, using the FFA transporter inhibitors or FAO pathway inhibitors could reverse the cancer cell secondary metabolic reprogramming. Apart from the metabolic reprogramming, the intrinsic genomic variability and heterogeneity of cancer cells are still the key players in tumor development [101]. The hypoxia environment strongly affected the unstable manner of cancer cells and triggered genomic mutation Therefore, the effect of anti-VEGF therapy was limited. In future study, we should further investigate the combination therapy effect of targeting cancer cells and the TME, for example, combine with TKI, FAO inhibitors and anti-VEGF in cancer therapy.

In Paper II, we provided an approach to increase the activation of the BAT through the megaBAT. Physiologically, the BAT dissipates FFA and produces heat to maintain our core body temperature in a cold environment. Because of this primary function of the BAT, increasing the activation of BAT could help our body burn down more fat depots. According

to a human study, the estimated 60g BAT in the supraclavicular area of adult human with fully activation may dissipate a huge amount of energy that is equivalent to approximately 4.0 kg of adipose tissue per year [90]. Therefore, in our study, we tried to enlarge the size of the BAT mass to dissipate more FFA in the whole body. However, unlike the WAT, which could change size in response to diet, exercise, or temperature [102], the size of BAT in adult human is stable [88]. Human infants have a large mass ratio of BAT, but this mass becomes tiny during human grew up. Therefore, we came up the idea about manipulating the stem cells of BAT may help to increase the tissue mass of BAT in adult. According to previous studies, the PDGFRα protein was considered as one of the progenitor cell markers of BAT [88,103,104]. Inhibiting the PDGFRα signaling pathway, we could induce more BAT progenitor cell differentiation. Later, by using a pharmacological approach and a specifically miR-485 to target the PDGFRα signaling in BAT, we successfully invented a method to enlarge the BAT into megaBAT. The megaBAT under cold exposure showed sufficient function to decrease the whole blood level FFA. However, in this study, we should also consider other population niches of BAT progenitor cells. There are approximately 10% of progenitor cells do not express PDGFRα. The roles of these cells in BAT differentiation deserve future study.

In addition, the pool of BAT progenitor cells is large, there are various niches of cells, with different functions [8,105]. In this study, we used different progenitor cell markers to distinguish them. Some of them have overlap expression markers, some of them do not. According to previous studies, these progenitor cells belong to the stromal tissues in BAT, and under other gatekeeper signals, they could differentiate into other stromal cells, including fibroblasts and muscle fibers [105,106]. In cancer scenarios, these multiple progenitor cells can contribute to produce cellular components in the TME. Therefore, targeting various gatekeeper signals to regulate progenitor cell differentiation may become a way to decrease stromal cellular components in cancer.

6.2.2 Reveal the roles of CAF and TAM in cancer growth and metastasis (Paper IV and V)

In our second topic, we revealed the roles of CAF and TAM in PDAC associated with cancer growth and metastasis, and the CAFs contributed to tumor metastasis in CR disruption host.

Compared to other types of cancer, IL-33 showed specifically high expression level in PDAC according to our results. It is known that IL-33 highly expresses in fibrotic tissue, for example pulmonary fibrosis [107]. The PDAC contains abundant fibroblasts and fibrotic structures. Our data supported IL-33 was expressed mainly in CAFs. Also, previous studies showed the dysregulation of IL-33/ST2 pathway mediates various inflammatory diseases, including atherosclerosis, asthma, inflammatory bowel disease (IBD), and type II diabetes [108]. Our results showed the expression level of ST2 was correlating with infiltrated macrophages in the PDAC tissues. According to other studies, the ST2 was expressed in various cell types, particularly the M2 macrophages [108]. Consistent with our results, the expression of CXCL3, which is the downstream protein of IL-33/ST2, had no overlap expression of M1 macrophage marker $iNOS^+$ but overlapped with the pan macrophage markers [109]. This finding indicated IL-33/ST2

induced M1 to M2 polarization. But since the ST2 has been found to show expression in various cell types, it is possible that CAFs cross talked with other cells, especially the immune cells, including neutrophils, regulatory T cells (Tregs) and mast cells, to trigger their downstream effects and resulted in fibrosis. Previously, researchers showed ST2 is expressed in innate lymphoid cell types (ILC2s) [110]. They found that IL-33 overstimulated ILC2 during tissue remodeling of the liver after chemical injury promoted liver fibrosis [108]. In this case, it is possible that CAFs utilizes the IL-33/ST2 signal interact with other cell types in the TME to form a fibrotic environment in the PDAC. This mechanism deserves further study.

Other researchers have discovered CXCL3-CXCR2 pathway induces cancer metastasis [111]. However, the mechanism is unclear. In our study, we used unbiased genomic analysis to demonstrate the CXCL3-CXCR2 induced a high collagen III expression. Collagen III, as one of the ECM members, plays a key role in cell adhesion and attachment [112]. With this glue-like protein, PDAC cancer cells stuck to CAFs and formed the cancer cell-CAFs cluster. These CXCL3-CXCR2 activated CAFs with myofibroblast phenotype, which showed as high α-SMA expression and elevated motility. Therefore, myofibroblasts hijacked the cancer cells into metastasis. Other studies had revealed how TAMs or CAFs clusters facilitate cancer cells metastasis. A study indicated that TAMs are the major cell types at the invasive edge between tumor cells and healthy mesenchymal tissue. The clusters of TAMs prevented the differentiation of antigen-presenting dendritic cells (DCs), which resulted in immunosuppression and facilitated cancer cell immune escape. Meanwhile, other studies have shown CXCL3 binds to CXCR2 on ECs and triggers angiogenesis, which could also promote cancer cell metastasis [113,114].

In another study, we revealed that CAFs were the key factors linking between CR disruption and cancer metastasis. CR disruption has been reported as a high risk for breast and prostate cancer [70]. Mechanistic studies indicated the CR core genes control the cell cycle, cell apoptosis, DNA damage repair and cancer cell metabolism [70]. Disruption of CR core genes contribute to the hallmarks of cancer. However, in our study, we discussed this issue from the cancer host aspect. Cancer-related sleeping disorders and insomnia are very common in cancer patients because of cancer pain [79]. Sleeping disorders and insomnia directly result in host CR disruption. Therefore, it is clinically relevant to understand the CR disruption of cancer host and its effects on cancer progression. Our study used a CR key gene *Bmal1* knockout (KO) mouse cancer model as a CR disruption host. Surprisingly, we found that tumor in *Bmal1* KO mice had a higher proliferation and metastatic rate. *Bmal1* gene transcriptionally targets to the *Serpine1* gene, which encodes PAI-1 protein. PAI-1 is the main regulator of the fibrinolytic system, negatively controlling its downstream proteins, including tPA and uPA. Lacking PAI-1 as an inhibitor of tPA and uPA, these proteins would accumulate in blood, triggering the plasminogen to plasmin conversion, as well as activation of plasmin-dependent matrix metalloproteinases (MMPs). As one of the activators of TGF-β, the accumulation of plasmin converted latent TGF-β to active TGF-β. Activated TGF-β promoted CAFs proliferation and transition of CAFs to myofibroblast. The myofibroblasts also clustered with cancer cells and promoted metastasis. With the zebrafish models inoculated with mouse cancer-CAFs co-

implantation as well as mouse co-implantation model, we found that CAFs from *Bmal1* KO cancer had a higher motility to promote cancer metastasis.

In this study, we described the link between CR disruption and cancer metastasis through the Bmal1-PAI-1-tPA/uPA-plasmin-TGF-β axis. It is possible that other factors involve in this scenario. In our results, we noticed tumors in the *Bmal1* KO mice demonstrated markedly hypoxia and angiogenesis, compared to the tumor in wt mice. The tissue hypoxia generated by accelerated angiogenesis, decreased pericyte vessel coverage and increased tumor cell proliferation led to high levels of HIF-1α and VEGF expression. It is possible that the high VEGF and hypoxia trigger tumor metastasis in the *Bmal1* KO mice. Also, studies found that the *Hif1a* and *Bmal1* have numerous co-targeted genes, for example, the *PER2* gene [70]. Hypoxia-induced elevated HIF-1α can occupy these gene promoters by non-canonical E-box region and increase their expression. In cancer, cancer cells produce high level of VEGF and HIF1α proteins, the accumulated HIF1α occupied the promoter of *PER2* gene and resulted in increased PER2 expression. As one of the negative feedback core genes in the CR transcription loop, high level of PER2 protein reversely inhibits *BMAL1* gene transcription. Because of core clock regulator role of *BMAL1* gene, the inhibition of *BMAL1* would result in other CR genes dysfunction. This provides a possible mechanism to explain tumor cells play a role in disrupting host CR, which could be the mechanism of sleeping disorders in patients with early-stage cancer [115].

7 CONCLUSIONS

In summary, this thesis represented the roles of various stromal cellular components and their function of the TME. By targeting the stomal cellular components, including CAAs, ECs, CAFs and TAMs, we succeed in resolving anti-VEGF-based AAD resistance in cancer with CAAs or lipid-rich environment; manipulating the BAT into megaBAT, which as a therapeutic tool of cancer and metabolic diseases; resolving FGF-2 high expressed cancer by depleting pericytes shield with combination therapy; revealing the link between CAFs and TAMs in PDAC metastasis and provide a therapeutic paradigm; revealing CAFs as the link between circadian rhythm disruption and cancer metastasis and provide a therapy approach.

8 ACKNOWLEDGEMENTS

During my doctoral study, I have received numerous helps from many people. Here I want to express my sincere thanks to all of you. Because of you, my study journey was full of fun and joy.

Professor Yihai Cao, my main supervisor. I would like to say I am forever grateful to you for you giving me the chance to come to Sweden and learning from you in five years ago. Your invitation totally changed my life. From the bottom of my heart, I really appreciate for your dedicated support and guidance to my research, as well as my life. Yes, I followed in your footsteps from scientific research to food recipes and gardening. In this five-year study, I learned many unique ways of thinking and conceiving about doing research from you. I will always keep what I have learned from you in my mind, and practise by my heart.

Dr. Kayoko Hosaka, my co-supervisor, I truly appreciate for your daily support and teaching in these five years. You are an amazing teacher and a good friend. We have a lot of joy and sour moments, leaving so many great memories. Thank you very much, my friend.

Dr. Takahiro Seki, thank you for your support in my projects. You are a great teacher and a good friend. I have learned a lot of rigorous research skills and attitude from you in scientific research. I will keep them in my mind.

Dr. Xu Jing, my co-first author, it is so happy to work with you every day. I have learned a lot from you. You are my fortune star. Thank you, my friend, my sister.

Dr. Qiqiao Du, my co-first author, it is so happy to meet you from the GEP program and worked together with you for two years in Sweden. I have learned a lot from you. Thank you, my friend, my brother.

Dr. Juan Gao, my young cousin, thank you for your support. It is so great to have you in Sweden with me.

Dr. Xingkang He, it was a coincidence of us came to Prof. Cao's lab on the same day. When I first came abroad, it was so great to have you here accompanying with me. Thank you, my friend.

Professor Yin Zhang, thank you so much for all your supports, my friend, my big brother.

Professor. Yunlong Yang and **Dr. Xiaoting Sun**, thank you so much for your supports in my Bmal1 project, I could not finish it without your assistance. I really appreciate that.

Professor. Lasse Jessen, thank you very much for your help in the start stage of my Bmal1 project. It was difficult. But I was so lucky to have you here, I could ask all the questions and always have your support. I truly appreciate that.

Professor. Laszlo Szekely, tremendous thanks for your supports in our COVID-19 project, I very appreciated that. It was so great to work and discuss with you about everything.

Here I also want to thank the financial support from the Oversea Study Program of Guangzhou Elite Project (GEP), Guangzhou, China and The First Affiliated Hospital of Guangzhou Medical University, China. I truly appreciate that.

I also want to thank my friends at Karolinska Institutet, including Dr. Ruining Liu, Dr. Keyi Geng, Dr. Yujie Zhang, Dr. Junjie Ma, Dr. Wenyu Li, Dr. Hua Chen and Dr. Dang Wei. It was really lucky to meet you guys in KI, thank you for all your supports and help.

At last, but the most importantly, I want to thank my family. It is your love and support accompany with me to accomplish this doctoral journey. Especially Andrew, my husband. I am really grateful for your love and support. I deeply appreciate you for those sweet little things you have done for me. They light me up every day. I love you.

感谢我的外婆对我的信任与支持。感谢我的父母对我的教养。感谢外公、爷爷、奶奶对我的养育，我永远怀念你们。感谢我所有的家人，是你们的爱与鼓励让我一路前行，永不畏惧。

9 REFERENCES

1 Anderson, N. M. & Simon, M. C. The tumor microenvironment. *Curr Biol* **30**, R921-r925, doi:10.1016/j.cub.2020.06.081 (2020).

2 Fukumura, D. & Jain, R. K. Tumor microenvironment abnormalities: causes, consequences, and strategies to normalize. *J Cell Biochem* **101**, 937-949, doi:10.1002/jcb.21187 (2007).

3 Nieman, K. M. *et al.* Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat Med* **17**, 1498-1503, doi:10.1038/nm.2492 (2011).

4 Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discov* **12**, 31-46, doi:10.1158/2159-8290.CD-21-1059 (2022).

5 Pani, G., Galeotti, T. & Chiarugi, P. Metastasis: cancer cell's escape from oxidative stress. *Cancer Metastasis Rev* **29**, 351-378, doi:10.1007/s10555-010-9225-4 (2010).

6 Quail, D. F. & Joyce, J. A. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* **19**, 1423-1437, doi:10.1038/nm.3394 (2013).

7 Thomas, D. & Radhakrishnan, P. Tumor-stromal crosstalk in pancreatic cancer and tissue fibrosis. *Mol Cancer* **18**, 14, doi:10.1186/s12943-018-0927-5 (2019).

8 Timmons, J. A. *et al.* Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc Natl Acad Sci U S A* **104**, 4401-4406, doi:10.1073/pnas.0610615104 (2007).

9 Dirat, B. *et al.* Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. *Cancer Res* **71**, 2455-2465, doi:10.1158/0008-5472.CAN-10-3323 (2011).

10 Kalluri, R. The biology and function of fibroblasts in cancer. *Nat Rev Cancer* **16**, 582-598, doi:10.1038/nrc.2016.73 (2016).

11 Ricella M Souza da Silva *et al.* Standardized Assessment of the Tumor-Stroma Ratio in Colorectal Cancer: Interobserver Validation and Reproducibility of a Potential Prognostic Factor. *Clinical pathology* **14**, 1-5 (2021).

12 Dagogo-Jack, I. & Shaw, A. T. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* **15**, 81-94, doi:10.1038/nrclinonc.2017.166 (2018).

13 Gunaydin, G. CAFs Interacting With TAMs in Tumor Microenvironment to Enhance Tumorigenesis and Immune Evasion. *Front Oncol* **11**, 668349, doi:10.3389/fonc.2021.668349 (2021).

14 Lee, P., Chandel, N. S. & Simon, M. C. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nature Reviews Molecular Cell Biology* **21**, 268-283, doi:10.1038/s41580-020-0227-y (2020).

15 Xie, S. *et al.* Dietary ketone body-escalated histone acetylation in megakaryocytes alleviates chemotherapy-induced thrombocytopenia. *Sci Transl Med* **14**, eabn9061, doi:10.1126/scitranslmed.abn9061 (2022).

16 Donaldson, M. S. Nutrition and cancer: a review of the evidence for an anti-cancer diet. *Nutr J* **3**, 19, doi:10.1186/1475-2891-3-19 (2004).

17 Xuan, W. *et al.* Circadian regulation of cancer cell and tumor microenvironment crosstalk. *Trends Cell Biol* **31**, 940-950, doi:10.1016/j.tcb.2021.06.008 (2021).

18 Prigerson, H. G. *et al.* Chemotherapy Use, Performance Status, and Quality of Life at the End of Life. *JAMA Oncology* **1**, 778-784, doi:10.1001/jamaoncol.2015.2378 (2015).

19 Zhao, X. *et al.* Nuclear receptors rock around the clock. *EMBO Rep* **15**, 518-528, doi:10.1002/embr.201338271 (2014).

20 Remon, J., Steuer, C. E., Ramalingam, S. S. & Felip, E. Osimertinib and other third-generation EGFR TKI in EGFR-mutant NSCLC patients. *Ann Oncol* **29**, i20-i27, doi:10.1093/annonc/mdx704 (2018).

21 Drilon, A. *et al.* Safety and Antitumor Activity of the Multitargeted Pan-TRK, ROS1, and ALK Inhibitor Entrectinib: Combined Results from Two Phase I Trials (ALKA-372-001 and STARTRK-1). *Cancer Discov* **7**, 400-409, doi:10.1158/2159-8290.Cd-16-1237 (2017).

22 Giantonio, B. J. *et al.* Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol* **25**, 1539-1544, doi:10.1200/jco.2006.09.6305 (2007).

23 Cohen, M. H., Gootenberg, J., Keegan, P. & Pazdur, R. FDA drug approval summary: bevacizumab plus FOLFOX4 as second-line treatment of colorectal cancer. *Oncologist* **12**, 356-361, doi:10.1634/theoncologist.12-3-356 (2007).

24 Mahoney, K. M., Freeman, G. J. & McDermott, D. F. The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma. *Clin Ther* **37**, 764-782, doi:10.1016/j.clinthera.2015.02.018 (2015).

25 Xiao, Y. & Yu, D. Tumor microenvironment as a therapeutic target in cancer. *Pharmacol Ther* **221**, 107753, doi:10.1016/j.pharmthera.2020.107753 (2021).

26 Yihai Cao & Langer, R. A review of Judah Folkman's remarkable achievements in biomedicine. *PNAS* **105**, 13203-13205 (2008).

27 Cao, Y. Tumor angiogenesis and molecular targets for therapy. *Frontiers in Bioscience* (2009).

28 Siemann, D. W. The unique characteristics of tumor vasculature and preclinical evidence for its selective disruption by Tumor-Vascular Disrupting Agents. *Cancer Treat Rev* **37**, 63-74, doi:10.1016/j.ctrv.2010.05.001 (2011).

29 Cao, Y. *et al.* Forty-year journey of angiogenesis translational research. *Sci Transl Med* **3**, 114rv113, doi:10.1126/scitranslmed.3003149 (2011).

30 Hosaka, K. *et al.* Tumour PDGF-BB expression levels determine dual effects of anti-PDGF drugs on vascular remodelling and metastasis. *Nat Commun* **4**, 2129, doi:10.1038/ncomms3129 (2013).

31 Ngambenjawong, C., Gustafson, H. H. & Pun, S. H. Progress in tumor-associated macrophage (TAM)-targeted therapeutics. *Adv Drug Deliv Rev* **114**, 206-221, doi:10.1016/j.addr.2017.04.010 (2017).

32 Blanco, R. & Gerhardt, H. VEGF and Notch in tip and stalk cell selection. *Cold Spring Harb Perspect Med* **3**, a006569, doi:10.1101/cshperspect.a006569 (2013).

33 Sewduth, R. & Santoro, M. M. "Decoding" Angiogenesis: New Facets Controlling Endothelial Cell Behavior. *Front Physiol* **7**, 306, doi:10.3389/fphys.2016.00306 (2016).

34 Gerhardt, H. & Betsholtz, C. Endothelial-pericyte interactions in angiogenesis. *Cell Tissue Res* **314**, 15-23, doi:10.1007/s00441-003-0745-x (2003).

35 De Palma, M., Biziato, D. & Petrova, T. V. Microenvironmental regulation of tumour angiogenesis. *Nat Rev Cancer* **17**, 457-474, doi:10.1038/nrc.2017.51 (2017).

36 Viallard, C. & Larrivee, B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. *Angiogenesis* **20**, 409-426, doi:10.1007/s10456-017-9562-9 (2017).

37 Cao, Y. Off-tumor target--beneficial site for antiangiogenic cancer therapy? *Nat Rev Clin Oncol* **7**, 604-608, doi:10.1038/nrclinonc.2010.118 (2010).

38 Lugano, R., Ramachandran, M. & Dimberg, A. Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol Life Sci* **77**, 1745-1770, doi:10.1007/s00018-019-03351-7 (2020).

39 LeBleu, V. S. & Kalluri, R. A peek into cancer-associated fibroblasts: origins, functions and translational impact. *Dis Model Mech* **11**, doi:10.1242/dmm.029447 (2018).

40 Arina, A. *et al.* Tumor-associated fibroblasts predominantly come from local and not circulating precursors. *Proc Natl Acad Sci U S A* **113**, 7551-7556, doi:10.1073/pnas.1600363113 (2016).

41 Kurashige, M. *et al.* Origin of cancer-associated fibroblasts and tumor-associated macrophages in humans after sex-mismatched bone marrow transplantation. *Commun Biol* **1**, 131, doi:10.1038/s42003-018-0137-0 (2018).

42 Bartoschek, M. *et al.* Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell RNA sequencing. *Nat Commun* **9**, 5150, doi:10.1038/s41467-018-07582-3 (2018).

43 Chen, Y., McAndrews, K. M. & Kalluri, R. Clinical and therapeutic relevance of cancer-associated fibroblasts. *Nat Rev Clin Oncol* **18**, 792-804, doi:10.1038/s41571-021-00546-5 (2021).

44 Feng, B., Wu, J., Shen, B., Jiang, F. & Feng, J. Cancer-associated fibroblasts and resistance to anticancer therapies: status, mechanisms, and countermeasures. *Cancer Cell Int* **22**, 166, doi:10.1186/s12935-022-02599-7 (2022).

45 Öhlund, D. *et al.* Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med* **214**, 579-596, doi:10.1084/jem.20162024 (2017).

46 Frödin, M. *et al.* Perivascular PDGFR-β is an independent marker for prognosis in renal cell carcinoma. *Br J Cancer* **116**, 195-201, doi:10.1038/bjc.2016.407 (2017).

47 Dong, H. *et al.* Overexpression of matrix metalloproteinase-9 in breast cancer cell lines remarkably increases the cell malignancy largely via activation of transforming growth factor beta/SMAD signalling. *Cell Prolif* **52**, e12633, doi:10.1111/cpr.12633 (2019).

48 Calon, A., Tauriello, D. V. & Batlle, E. TGF-beta in CAF-mediated tumor growth and metastasis. *Semin Cancer Biol* **25**, 15-22, doi:10.1016/j.semcancer.2013.12.008 (2014).

49 Maxwell, P. H. *et al.* Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 8104-8109, doi:DOI 10.1073/pnas.94.15.8104 (1997).

50 Yamauchi, M., Barker, T. H., Gibbons, D. L. & Kurie, J. M. The fibrotic tumor stroma. *J Clin Invest* **128**, 16-25, doi:10.1172/JCI93554 (2018).

51 Cheng, Y. *et al.* Cancer-associated fibroblasts induce PDL1+ neutrophils through the IL6-STAT3 pathway that foster immune suppression in hepatocellular carcinoma. *Cell Death Dis* **9**, 422, doi:10.1038/s41419-018-0458-4 (2018).

52 Li, X. *et al.* Single-cell RNA sequencing reveals a pro-invasive cancer-associated fibroblast subgroup associated with poor clinical outcomes in patients with gastric cancer. *Theranostics* **12**, 620-638, doi:10.7150/thno.60540 (2022).

53 Colangelo, T. *et al.* Friend or foe? The tumour microenvironment dilemma in colorectal cancer. *Biochim Biophys Acta Rev Cancer* **1867**, 1-18, doi:10.1016/j.bbcan.2016.11.001 (2017).

54 Liu, T., Zhou, L., Li, D., Andl, T. & Zhang, Y. Cancer-Associated Fibroblasts Build and Secure the Tumor Microenvironment. *Front Cell Dev Biol* **7**, 60, doi:10.3389/fcell.2019.00060 (2019).

55 Liu, T. *et al.* Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. *J Hematol Oncol* **12**, 86, doi:10.1186/s13045-019-0770-1 (2019).

56 Melisi, D. *et al.* Safety and activity of the TGFβ receptor I kinase inhibitor galunisertib plus the anti-PD-L1 antibody durvalumab in metastatic pancreatic cancer. *J Immunother Cancer* **9**, doi:10.1136/jitc-2020-002068 (2021).

57 Cao, Y. Adipocyte and lipid metabolism in cancer drug resistance. *J Clin Invest* **129**, 3006-3017, doi:10.1172/JCI127201 (2019).

58 Lengyel, E., Makowski, L., DiGiovanni, J. & Kolonin, M. G. Cancer as a Matter of Fat: The Crosstalk between Adipose Tissue and Tumors. *Trends Cancer* **4**, 374-384, doi:10.1016/j.trecan.2018.03.004 (2018).

59 Koundouros, N. & Poulogiannis, G. Reprogramming of fatty acid metabolism in cancer. *Br J Cancer* **122**, 4-22, doi:10.1038/s41416-019-0650-z (2020).

60 Zaidi, N. *et al.* Lipogenesis and lipolysis: the pathways exploited by the cancer cells to acquire fatty acids. *Prog Lipid Res* **52**, 585-589, doi:10.1016/j.plipres.2013.08.005 (2013).

61 Rodriguez-Carrio, J. *et al.* Free Fatty Acids Profiles Are Related to Gut Microbiota Signatures and Short-Chain Fatty Acids. *Front Immunol* **8**, 823, doi:10.3389/fimmu.2017.00823 (2017).

62 Ackerman, D. & Simon, M. C. Hypoxia, lipids, and cancer: surviving the harsh tumor microenvironment. *Trends Cell Biol* **24**, 472-478, doi:10.1016/j.tcb.2014.06.001 (2014).

63 Arango Duque, G. & Descoteaux, A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol* **5**, 491, doi:10.3389/fimmu.2014.00491 (2014).

64 Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J. & Hill, A. M. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* **164**, 6166-6173, doi:10.4049/jimmunol.164.12.6166 (2000).

65 Pathria, P., Louis, T. L. & Varner, J. A. Targeting Tumor-Associated Macrophages in Cancer. *Trends Immunol* **40**, 310-327, doi:10.1016/j.it.2019.02.003 (2019).

66 Noy, R. & Pollard, J. W. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* **41**, 49-61, doi:10.1016/j.immuni.2014.06.010 (2014).

67 Nakamura, K. & Smyth, M. J. Myeloid immunosuppression and immune checkpoints in the tumor microenvironment. *Cell Mol Immunol* **17**, 1-12, doi:10.1038/s41423-019-0306-1 (2020).

68 Wang, J., Li, D., Cang, H. & Guo, B. Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. *Cancer Med* **8**, 4709-4721, doi:10.1002/cam4.2327 (2019).

69 Masri, S. & Sassone-Corsi, P. The emerging link between cancer, metabolism, and circadian rhythms. *Nat Med* **24**, 1795-1803, doi:10.1038/s41591-018-0271-8 (2018).

70 Shafi, A. A. & Knudsen, K. E. Cancer and the Circadian Clock. *Cancer Res* **79**, 3806-3814, doi:10.1158/0008-5472.CAN-19-0566 (2019).

71 Takahashi, J. S. Transcriptional architecture of the mammalian circadian clock. *Nat Rev Genet* **18**, 164-179, doi:10.1038/nrg.2016.150 (2017).

72 Panda, S., Hogenesch, J. B. & Kay, S. A. Circadian rhythms from flies to human. *Nature* **417**, 329-335, doi:10.1038/417329a (2002).

73 Sillen, M. & Declerck, P. J. Targeting PAI-1 in Cardiovascular Disease: Structural Insights Into PAI-1 Functionality and Inhibition. *Front Cardiovasc Med* **7**, 622473, doi:10.3389/fcvm.2020.622473 (2020).

74 Jensen, L. D. & Cao, Y. Clock controls angiogenesis. *Cell Cycle* **12**, 405-408, doi:10.4161/cc.23596 (2013).

75 Parascandolo, A. *et al.* Effect of naive and cancer-educated fibroblasts on colon cancer cell circadian growth rhythm. *Cell Death Dis* **11**, 289, doi:10.1038/s41419-020-2468-2 (2020).

76 Izumi, H., Wang, K., Morimoto, Y., Sasaguri, Y. & Kohno, K. Circadian disruption and cancer risk: a new concept of stromal niche (review). *Int J Oncol* **44**, 364-370, doi:10.3892/ijo.2013.2201 (2014).

77 Masri, S. *et al.* Lung Adenocarcinoma Distally Rewires Hepatic Circadian Homeostasis. *Cell* **165**, 896-909, doi:10.1016/j.cell.2016.04.039 (2016).

78 Blacher, E. *et al.* Aging disrupts circadian gene regulation and function in macrophages. *Nat Immunol* **23**, 229-236, doi:10.1038/s41590-021-01083-0 (2022).

79 Ruan, W., Yuan, X. & Eltzschig, H. K. Circadian rhythm as a therapeutic target. *Nat Rev Drug Discov* **20**, 287-307, doi:10.1038/s41573-020-00109-w (2021).

80 Tol, J. *et al.* Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med* **360**, 563-572, doi:10.1056/NEJMoa0808268 (2009).

81 Miller, K. D. *et al.* Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. *J Clin Oncol* **23**, 792-799, doi:10.1200/jco.2005.05.098 (2005).

82 Van Cutsem, E. *et al.* Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* **27**, 2231-2237, doi:10.1200/jco.2008.20.0238 (2009).

83 Kung, A. L., Wang, S., Klco, J. M., Kaelin, W. G. & Livingston, D. M. Suppression of tumor growth through disruption of hypoxia-inducible transcription. *Nature Medicine* **6**, 1335-1340, doi:Doi 10.1038/82146 (2000).

84 DeBerardinis, R. J. & Chandel, N. S. We need to talk about the Warburg effect. *Nat Metab* **2**, 127-129, doi:10.1038/s42255-020-0172-2 (2020).

85 Vander Heiden, M. G., Cantley, L. C. & Thompson, C. B. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* **324**, 1029-1033, doi:10.1126/science.1160809 (2009).

86 Seki, T. *et al.* Brown-fat-mediated tumour suppression by cold-altered global metabolism. *Nature* **608**, 421-428, doi:10.1038/s41586-022-05030-3 (2022).

87 Urisarri, A. *et al.* BMP8 and activated brown adipose tissue in human newborns. *Nat Commun* **12**, 5274, doi:10.1038/s41467-021-25456-z (2021).

88 Zoico, E. *et al.* Brown and Beige Adipose Tissue and Aging. *Front Endocrinol (Lausanne)* **10**, 368, doi:10.3389/fendo.2019.00368 (2019).

89 Institute, N. C. *Angiogenesis Inhibitors*, <https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/angiogenesis-inhibitors-fact-sheet> (

90 Virtanen, K. A. *et al.* Functional brown adipose tissue in healthy adults. *N Engl J Med* **360**, 1518-1525, doi:10.1056/NEJMoa0808949 (2009).

91 Zhang, J. *et al.* Differential roles of PDGFR-alpha and PDGFR-beta in angiogenesis and vessel stability. *FASEB J* **23**, 153-163, doi:10.1096/fj.08-113860 (2009).

92 Piersma, B., Hayward, M. K. & Weaver, V. M. Fibrosis and cancer: A strained relationship. *Biochim Biophys Acta Rev Cancer* **1873**, 188356, doi:10.1016/j.bbcan.2020.188356 (2020).

93 Lindner, T. *et al.* Targeting of activated fibroblasts for imaging and therapy. *EJNMMI Radiopharm Chem* **4**, 16, doi:10.1186/s41181-019-0069-0 (2019).

94 Liu, C. *et al.* A Zebrafish Model Discovers a Novel Mechanism of Stromal Fibroblast-Mediated Cancer Metastasis. *Clin Cancer Res* **23**, 4769-4779, doi:10.1158/1078-0432.CCR-17-0101 (2017).

95 Carmona, P., Mendez, N., Ili, C. G. & Brebi, P. The Role of Clock Genes in Fibrinolysis Regulation: Circadian Disturbance and Its Effect on Fibrinolytic Activity. *Front Physiol* **11**, 129, doi:10.3389/fphys.2020.00129 (2020).

96 Schoenhard, J. Regulation of the PAI-1 promoter by circadian clock components: differential activation by BMAL1 and BMAL2. *Journal of Molecular and Cellular Cardiology* **35**, 473-481, doi:10.1016/s0022-2828(03)00051-8 (2003).

97 Yang, Y. & Cao, Y. The impact of VEGF on cancer metastasis and systemic disease. *Semin Cancer Biol* **86**, 251-261, doi:10.1016/j.semcancer.2022.03.011 (2022).

98 Bergers, G. & Hanahan, D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* **8**, 592-603, doi:10.1038/nrc2442 (2008).

99 Lee, J. W., Ko, J., Ju, C. & Eltzschig, H. K. Hypoxia signaling in human diseases and therapeutic targets. *Exp Mol Med* **51**, 1-13, doi:10.1038/s12276-019-0235-1 (2019).

100 Baran, N. & Konopleva, M. Molecular Pathways: Hypoxia-Activated Prodrugs in Cancer Therapy. *Clin Cancer Res* **23**, 2382-2390, doi:10.1158/1078-0432.CCR-16-0895 (2017).

101 Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646-674, doi:10.1016/j.cell.2011.02.013 (2011).

102 Parlee, S. D., Lentz, S. I., Mori, H. & MacDougald, O. A. Quantifying size and number of adipocytes in adipose tissue. *Methods Enzymol* **537**, 93-122, doi:10.1016/b978-0-12-411619-1.00006-9 (2014).

103 Sun, C. *et al.* Mosaic Mutant Analysis Identifies PDGFRα/PDGFRβ as Negative Regulators of Adipogenesis. *Cell Stem Cell* **26**, 707-721.e705, doi:10.1016/j.stem.2020.03.004 (2020).

104 Berry, R. & Rodeheffer, M. S. Characterization of the adipocyte cellular lineage in vivo. *Nat Cell Biol* **15**, 302-308, doi:10.1038/ncb2696 (2013).

105 Schulz, T. J. *et al.* Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc Natl Acad Sci U S A* **108**, 143-148, doi:10.1073/pnas.1010929108 (2011).

106 Schulz, T. J. & Tseng, Y. H. Brown adipose tissue: development, metabolism and beyond. *Biochem J* **453**, 167-178, doi:10.1042/BJ20130457 (2013).

107 Di Carmine, S., Scott, M. M., McLean, M. H. & McSorley, H. J. The role of interleukin-33 in organ fibrosis. *Discovery Immunology* **1**, doi:10.1093/discim/kyac006 (2022).

108 Griesenauer, B. & Paczesny, S. The ST2/IL-33 Axis in Immune Cells during Inflammatory Diseases. *Front Immunol* **8**, 475, doi:10.3389/fimmu.2017.00475 (2017).

109 McNeill, E. *et al.* Regulation of iNOS function and cellular redox state by macrophage Gch1 reveals specific requirements for tetrahydrobiopterin in NRF2 activation. *Free Radic Biol Med* **79**, 206-216, doi:10.1016/j.freeradbiomed.2014.10.575 (2015).

110 Li, D. *et al.* IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. *J Allergy Clin Immunol* **134**, 1422-1432.e1411, doi:10.1016/j.jaci.2014.05.011 (2014).

111 Liao, W. *et al.* KRAS-IRF2 Axis Drives Immune Suppression and Immune Therapy Resistance in Colorectal Cancer. *Cancer Cell* **35**, 559-572 e557, doi:10.1016/j.ccell.2019.02.008 (2019).

112 Xu, S. *et al.* The role of collagen in cancer: from bench to bedside. *J Transl Med* **17**, 309, doi:10.1186/s12967-019-2058-1 (2019).

113 De la Fuente, M., MacDonald, T. T. & Hermoso, M. A. The IL-33/ST2 axis: Role in health and disease. *Cytokine Growth Factor Rev* **26**, 615-623, doi:10.1016/j.cytogfr.2015.07.017 (2015).

114 Kakkar, R. & Lee, R. T. The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nat Rev Drug Discov* **7**, 827-840, doi:10.1038/nrd2660 (2008).

115 Ancoli-Israel, S. Sleep Disturbances in Cancer: A Review. *Sleep Med Res* **6**, 45-49, doi:10.17241/smr.2015.6.2.45 (2015).