Cancer–stroma interactions as a target for cancer treatment

I. V. Alekseenko[1,2], V. V. Pleshkan[1,2], E. D. Sverdlov[1,2]

[1] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS
  16/10, Miklukho-Maklaya, Moscow, Russian Federation, 117997

[2] Institute of Molecular Genetics, Russian Academy of Sciences,
  2, Kurchatov Sq. Moscow Russian Federation, 123182
  irina.alekseenko@mail.ru

During tumor evolution, cancer cells use the tumor-stroma crosstalk to reorganize the microenvironment for maximum robustness of tumor. The success of immune checkpoint therapy generates a new cancer therapy paradigm: an effective cancer treatment should not aim to influence the individual components of super complex intracellular interactomes (molecular targeting), but rather to disrupt the intercellular interactions between cancer and stromal cells, thus breaking the tumor as a whole. In this minireview we consider cancer associated fibroblasts (CAF) and their interactions with cancer cells as a promising direction for cancer therapy.

K e y w o r d s: cancer, hallmark, therapy, immunotherapy, stroma, crosstalk

Abbreviations: CAF — cancer associated fibroblast; ECM — extracellular matrix; TCR — T cell receptor; TIL — tumor-infiltrating lymphocyte; TME –tumor microenvironment.

Introduction. Not only Cancer cells but their microenvironment is critical for tumor progression

In 2000, Hanahan *et al.* commented that the medical implications of the concept of common hallmarks of cancer are as follows: "We envision development of anticancer drugs targeted to each of the hallmark capabilities of cancer; some, used in appropriate combinations … will be able to prevent incipient cancers from developing, while others will cure preexisting cancers, elusive goals at present." [1]. The hallmarks were principally deduced for cancer cells, [2, 3], although almost all of them, except replicative immortality, which is questionable, implicated the participation of the tumor microenvironment cells [2]. Therefore, the concept in this approach implies that the therapies act against cancer cells. As early as 2006, Orimo and Weinberg noted the importance of stroma for tumor progression [4]. From approximately 2010, the number of publications describing stroma's contribution

to cancer development has quickly increased [2, 5, 6], although "abetting microenvironment" has been included in the list of the main hallmarks only in 2017 [7].

This inclusion makes sense. The current view of the tumor stroma is not just a physical support of mutated epithelial cells. All tumors engage in a broad repertoire of normal cells in their evolution and adopt them for their needs. The recruited normal cells facilitate the acquisition of characteristic traits and form what is called the tumor microenvironment (TME). TME is an ecological niche, which plays the most important role in both the development of a primary tumor and its metastasizing [2, 8–13].

Neither cancer cells, nor stromal cells alone, but their interactions lead to the evolution of a tumor as an organ-like entity. These interactions include: (i) direct binary contacts between ligands and receptors exposed on the surfaces of cancer and stroma cells, and (ii) paracrine communications between cancer (usually epithelial) cells and various cells of TME [14, 15] (Fig. 1). Some authors use the term "symbiotic" [16, 17] for tumor–stroma interaction: "The relationship between tumor and stroma is symbiotic. Stromal cells are corrupted by malignant epithelium, creating a permissive microenvironment, which drives cancer progression" [18] (see also [16, 17]).

It is now clear that to defeat cancer, we should move from the indecipherable complexity of intracellular interactomes to disrupting the system as a whole by destroying interactions of its parts.

This simple "home-grown intuition" [19] determines a new paradigm for cancer therapy: the search and destruction of the intercellular crosstalk that lies at the root of the success of the malignant tumors' murderous mission.

A stromal component of tumors — an indispensable part of cancer evolution

The American National Cancer Institute defines TME as "normal cells, molecules and blood vessels that surround and feed a tumor. A tumor can change its microenvironment; the microenvironment can affect how a tumor grows and spreads." In solid tumors, the cancer microenvironment consists of two main components, cellular and non-cellular, whose ratios and composition vary depending on the location and stage of the tumor.

The non-cellular components mainly include the extracellular matrix (ECM) composed of proteins, glycoproteins, and proteoglycans, which serves as a scaffold for supporting tissue architecture [2, 8, 20].

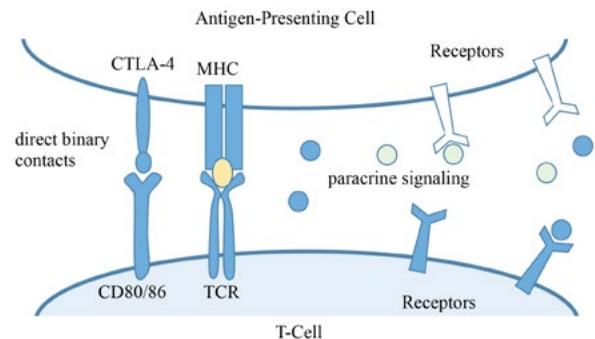The cellular components include fibroblasts, such as cancer associated fibroblasts (CAFs),

Fig. 1. Direct and paracrine interactions in tumor. Direct binary contacts between antigen-presenting cell and T-cell are displayed in example of MHC-antigene-Tcell receptor (TCR) and checkpoint molecules (CTLA-4 and CD80/86) interactions. Paracrine signaling is presented by soluble factors (various circles) and their receptors.

mesenchymal stem cells, adipocytes, pericytes, endothelial cells, networks of lymphatic vessels, and tumor-infiltrating cells of the immune system [18, 21–24]. From the therapeutic point of view, immune cell interactions with cancer cells might be the most successful targets for cancer treatment, and they could serve as a paradigm for more general approach.

Immune checkpoint therapy — a new paradigm for tumor therapy

T-cells of the immune system have proteins on their surface called checkpoints that turn on an immune response and other proteins that turn it off. Checkpoint proteins activate T-cells, for example, when an infection or cancer cells is present. However, if T-cells are active for too long, or react to things they shouldn't, then other checkpoints switch off the T-cells. Some cancer cells make high levels of checkpoint proteins that switch off T-cells, so that they can no longer recognize and kill cancer cells.

The simple principle of how the T-cells can avoid immunosuppression and resume tumor annihilation is illustrated in Fig. 2. Monoclonal antibodies against CTLA-4 or PD-1, or their ligands, disrupt the interaction of these molecules with T-cells allowing them to destroy tumors. This concept was proven by a revolutionary therapeutic success of targeting the binary interactions between the stromal immune cells and antigen-presenting cells, stromal immune cells and cancer cells, stromal immune cells (CD8+ cytotoxic lymphocytes) and CAFs. This kind of therapy was named the immune checkpoint therapy.

The most impressive effect of the CTLA-4 blockade is its ability to induce a long-term tumor regression that lasted up to 13 years in clinical trials with some melanoma patients. However, success rate in the case of melanoma was only about 8 % (see the latest data in [25]). Moreover, drug-activated T-cells affect healthy tissues. Clinical trials revealed severe side effects in about 15 % of patients, including several fatal outcomes. The reader can find the toxicity data in [26]. Still, the inhibition of CTLA-4 checkpoint made a revolutionary shift in the perception of cancer as an incurable disease. The success of immunotherapy stimulated the search for other inhibiting checkpoints for cancer treatment [27, 28].
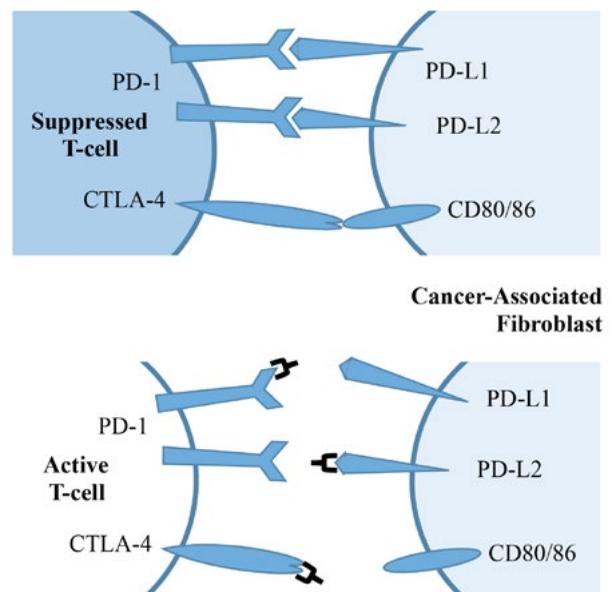

Fig. 2. Suppression of T-cell and its activation by checkpoint inhibitors. On the upper side the T-cell is suppressed by expressed on CAF surface ligands PD-L1/PD-2 and CD80/86 binding to PD-1 and CTLA-4 receptors of the T-cell, respectively. Lower is demonstrated restoration of T-cell activity when blocking antibodies (black ancipital fork) to various receptors/ligands are present. This disrupts the cell-cell interaction.

273

CTLA-4 and PD-1 regulate different inhibiting pathways and have the non-overlapping action mechanisms, suggesting that a combined therapy might be more efficient. Indeed, this was experimentally demonstrated in preclinical trials with mouse models. The preliminary clinical trials with anti -CTLA-4 combined with anti-PD-1 or anti-PD-L1 antibodies in other types of tumors produced promising results that declare the new combination immunotherapy an efficient strategy for cancer patients [27, 29]. However, the combined procedure has a somewhat higher toxicity.

Although these methods have greatly increased the lifespan of many patients with malignant neoplasms, many patients with common cancer types do not respond to this treatment. Further, inhibition of immune checkpoints causes multiple side effects, mostly autoimmune inflammatory reactions also known as immune-related adverse events (IRAEs) [26, 30–32].

Lessons of checkpoint therapy. Intercellular (possibly, synapse-like) contacts vs intracellular interactomes

Cell-surface proteins represent attractive targets for therapy due to their accessibility and involvement in essential signaling pathways, often dysregulated in cancer [33]. A receptor-ligand *interaction* is in itself a single key event — the binding of a signaling molecule (ligand) to its receiving molecule (receptor). Thus, they are involved in relatively simple binary interactions.

This is the basis of well-recognized druggable properties of receptors and their cognate ligands, which make them especially useful

clinical targets [34]. Furthermore, interacting cells in intercellular contacts are brought together to a distance comparable to the length of the receptor-ligand complexes, typically 15-40 nm [35]. Therefore, inhibition of the two targets might also result in the inhibition of paracrine crosstalk.

These considerations lead to a concept of therapeutically promising area of direct intercellular interactions as an antithesis of molecular-targeted therapy whose targets are the components of complex intracellular interactomes. Immune checkpoint therapy is a striking example of the success of the above-mentioned concept [36]. However, its complexity is manifested here by its rather high toxicity and the enormous variability of patients' responses ranging from none to complete remission, which presents a challenging problem [26, 30, 37, 38].

Worse still, the available long-term follow-up data on melanoma shows that a substantial number of patients that were earlier responding to the therapy with inhibitors of immune checkpoints become resistant [38, 39]. We do not understand why **T-cell checkpoints are ineffective in the majority of cancer patients**. This could be because their immune system does not recognize antigens of cancer cells or due to different mechanisms of immune inhibition [40].

A multitude of new agents targeting other immune and non-immune processes and tumor components is under investigation [39]. These include inhibitors of immune checkpoints, co-stimulating agonists, oncolytic viruses, vaccines, and adoptive cell therapy, as well as combinations with traditional methods of treatment [41].

Other TME components as potential participants of cancer stroma interaction

Keeping in mind a successful approach of destroying the direct interactions between immune and cancer cells, we hypothesize that a similar strategy might be fruitful if such pro-tumor binary contacts existed between the cancer cells and other components of stroma. It is widely accepted that paracrine crosstalk between tumor stroma cells causes a transformation of stromal fibroblasts to CAFs. The binary contacts between cancer cells and other components of stroma might be a target for therapeutic action. We will give a very concise outline of the potentially promising exploratory approaches wherein tumor-stroma and stroma-stroma interactions can be detected. To this end, we will consider an example of CAFs which are better studied than the other stromal constituents.

A brief overview of cancer-associated fibroblasts, barely explored architects of cancer pathogenesis

CAFs are some of the most prevalent stromal cells in a number of carcinomas, including breast, prostate, pancreas, esophagus, and intestine cancer [22]. In other carcinomas, including ovarian carcinoma, melanomas, and kidney tumors, CAFs are less frequent, but still occur [8]. CAFs as targets for enhancing cancer therapy efficiency attracted great attention. Some authors even call them "The Architects of Stroma Remodeling" [42] or "Architects of Cancer Pathogenesis" [43]. **CAFs have been reported to variously affect the tumor progression, involving ECM degradation, release of numerous soluble factors, regulation of tumor metabolism, and promotion of cancer cell proliferation, migration, and metastasis**. The most recent findings are found in the relevant reviews [22–24, 42, 44, 45].

The normal fibroblasts can have a variety of suppressive functions against the initiation of cancer and metastatic cells through direct contacts with cells and **paracrine signaling with soluble factors**. The tumor-induced transformation of the normal fibroblasts into CAFs causes a number of pro-tumorigenic signals, followed by a distortion of the normal tissue structure, thus supporting the growth of cancer cells [46]. CAFs are a heterogeneous 'family' or 'group' of cells that exhibit mesenchymal-like features.

Conversion of the normal fibroblasts to CAFs is considered a three-step process. First, distant normal cells are recruited by malignant or pre-malignant cells through **paracrine and endocrine signals**. Second, the recruited cells are **transformed** into CAFs. Finally, the third step is the **maintenance, expansion and evolution of CAF** populations in the cancer microenvironment, enabled by the persistent signals produced by malignant cells [47, 48]. In return, CAF population emanates paracrine signals that affect cancer progression. Bidirectional crosstalk between cancer cells and fibroblasts is presumed to be the leading cause of malignant cancer phenotype formation [49, 50].

One of the most significant features of CAFs is that their phenotype, which promotes tumor progression, is **stably maintained *in vitro* and *ex vivo* even without a steady contact with neighboring cancer cells** [20, 45, 51]. Recent studies reported that many types of cells could be recruited as predecessors of CAFs: resident

tissue fibroblasts, peritumoral adipocytes, bone marrow mesenchymal stem cells, hematopoietic stem cells, and many others [44, 45]. After recruiting from various sources, a subset of these precursors acquires the CAFs phenotype through complex activation processes that are still poorly understood. Most researches agree that irrespective of the precursor, CAFs express similar sets of markers, such as α-smooth muscle actin (α-SMA), fibroblast activation protein (FAP), and the α and β platelet-derived growth factor receptor (PDGFR) [44]. Unlike in epithelial cancer cells, the **genetic changes such as oncogene/tumor suppressor mutations are rare in CAFs**. In contrast, **epigenetic changes**, such as DNA methylation, histone modifications and nucleosome structure, changes in the expression of non-coding RNAs and abnormal activation of several signaling pathways, **are often observed when the CAF phenotype is acquired**. These changes affect the expression of many genes encoding growth factors, cytokines, and other products which intensifies proliferation, stimulates secretion of ECM proteins and various growth factors, and causes remodeling of cytoskeleton [2, 8, 22, 44, 45, 52].

Therefore, the stroma currently attracts a significant attention of researchers developing the new approaches to cancer treatment [5, 21, 51, 53].

Cancer associated fibroblasts can inhibit antitumor immune response through direct contact with immune cells

Because of their preponderance in the tumor microenvironment, CAFs were recently studied as regulators of immune cell recruitment and function. As the result, **CAFs were shown to play pro-inflammatory and immunosuppressive roles through secretion of TGF and other cytokines, thus affecting both the innate and adaptive immune response** [45, 54]. In this review, we will consider direct contact of CAFs with cells of the immune system, which, in our opinion, are important for strengthening and guiding the action of paracrine factors.

CAFs can establish direct contacts with immune cells and affect the efficiency of checkpoint immunotherapy by means of the expression of co-inhibitory receptor ligands [55–58]). By now, such a possibility was experimentally demonstrated for PD-L1 and/or PD-L2 expression. Nazareth and colleagues [57] found a constitutively high expression of functional PD-L1 and 2 in the fibroblasts cultured from human non-small cell lung cancers. It was also shown that CAFs of large intestine cancer express PD-L1 and PD-L2 and negatively regulate the proliferative response of CD4$^+$ Th-cells. Similar observations were reported for CAFs from melanoma cells (see review [45]). However, most of these findings were made in *in vitro* experiments using isolated CAFs, and, therefore, require further studies to confirm the physiological significance of PD-L1/L2 expression by CAFs for their immunosuppressive role *in vivo* [45].

Recent research [55], presents further evidence of the immuno-inhibiting function of CAFs resulting from their **direct interactions with immune cells**. The authors show that CAFs can function as antigen presenting cells, able to absorb, process, and present on their surface tumor specific antigens combined with MHC-I proteins. With the help of PD-L2 and

FASL, this triggers an antigen-specific negative regulation of tumor-specific CD8+T cells, which leads to their dysfunction and apoptosis. Neutralization of PD-L2 or FASL reactivates the cytotoxic capacity of T cells *in vitro* and *in vivo*.

Thus, CAFs might support T-cell suppression within the tumor microenvironment by a mechanism dependent on immune checkpoint activation. [55], making it another mechanism of **T-cell depletion** and dysfunction within tumors [55].

CAFs can directly interact with cancer cells and enhance their invasion and metastasis

CAFs are often found in the vicinity of, or in direct contact with, neoplastic cells [8, 22, 23, 53]. However, only a **few reports provide an experimental evidence for the CAF-cancer cell direct interaction and study its functional consequences**. The most obvious and important consequence of such direct interactions is the involvement of **CAFs in promoting cancer cell epithelial-mesenchymal transition**, invasion and metastasis [42, 59–64]. This should be expected as collective cell migration is ubiquitous in multicellular organisms. In addition, it is recognized that the physical interaction between cells in conjunction with chemical signals plays a fundamental role in this process [65].

Gaggioli *et al.* [59] demonstrated that CAFs led the invasion of squamous cell carcinoma cells (SCCs) by generating tracks in the extracellular matrix in a co-culture system. During joint invasion, the leading cells were CAFs, and associated SCC cells followed. Thus, SCC cell invasion needs either close proximity, or

direct contact, to CAFs. Similar evidence is presented in the review [63].

To investigate the differential contribution of direct cell–cell contacts and paracrine signaling factors to NSCLC metastasis, Choe *et al.* [61] performed two types of co-cultures: direct co-cultures of the NSCLC cell line with primary cultures of CAFs from patients with resected NSCLC and indirect cocultures across a separable membrane. CAFs **more potently induced EMT in case of direct co-culture,** providing evidence that the physical contacts between NSCLC cells and CAFs might control the metastatic potential of NSCLC. This probably does not exclude the participation of paracrine crosstalk that could be strengthened by the physical cell-to-cell interaction, similar to the immune synapses.

In a more recent review [42], it is indicated that CAFs adjacent to cancer regions were able to increase the invasiveness of cancer cells through both cell-cell interactions and various pro-invasive molecules, such as cytokines, chemokines and inflammatory mediators. It is also known [42] that CAFs can travel together in blood with circulating murine metastatic lung carcinoma cancer cells probably supporting the cancer cell viability and growth advantage at the metastatic site. The authors hypothesized that in invasive tumors, the cancer and stromal cells were in direct contact and established a complex crosstalk that evolved during tumor development.

In a very important study [64], the authors demonstrated that CAFs caused a collective invasion by means of a heterophilic adhesion involving N-cadherin at the CAF membrane and E-cadherin at the cancer cell membrane. Impairment of the E-cadherin/N-cadherin ad-

hesion abrogates the ability of CAFs to guide collective cell migration and blocks cancer cell invasion. In parallel, the organizers of intercellular junctions, nectins and afadin, are recruited to the cancer cell/CAF interface. These findings show that a mechanically active heterophilic adhesion between CAFs and cancer cells enables cooperative tumor invasion. Contacts between cancer cells and CAFs may also be implemented through the interaction of Eph-receptor and reciprocal ephrin ligands [66]. One can assume that these direct contacts form synapse-like structures, strengthening the paracrine cross-talk.

CAFs promote tumor invasion and metastasis. We show that CAFs exert a physical force on cancer cells that enables their collective invasion. Force **transmission is mediated by a heterophilic adhesion involving N-cadherin at the CAF membrane and E-cadherin at the cancer cell membrane.** This adhesion is mechanically active. When subjected to force, it triggers β-catenin recruitment and adhesion reinforcement dependent on α-catenin/vinculin interaction. Impairment of **E-cadherin/N-cadherin adhesion abrogates the ability of CAFs to guide collective cell migration and blocks cancer cell invasion**. N-cadherin also mediates repolarization of the CAFs away from the cancer cells. In parallel, nectins and afadin are recruited to the cancer cell/CAF interface and CAF repolarization is afadin dependent. Heterotypic junctions between CAFs and cancer cells are observed in patient-derived material. Together, our findings show that a mechanically active heterophilic adhesion between CAFs and cancer cells enables cooperative tumour invasion [64].

Attempts of targeting the interaction between CAFs and carcinoma cells

The sinister role of direct interactions of CAFs with cancer cells in the process of metastasis makes it especially important to destroy these contacts for therapeutic purposes. With such a goal, Yamaguchi *et al.* [63] tried to identify **inhibitors of direct interaction between CAFs and cancer cells**, and found that the Src inhibitor dasatinib effectively blocked the physical association between CAFs and scirrhous gastric carcinoma (SGC) cells with a very low cytotoxic effect. Dasatinib was also effective against peritoneal dissemination of SGC in mouse model experiments. Importantly, histological analysis revealed that metastasizing tumors were less associated with stromal fibroblasts in mice treated with dasatinib compared to controls. These results demonstrate that **direct interaction between CAFs and SGC cells can be a target for anti-metastasis therapy** [63]. Nevertheless, the authors advise caution, referencing the studies which showed that the depletion of CAFs in mouse models accelerated progression of pancreatic cancer. Although these results are contradictory, they accentuate the need for thorough safety testing of the inhibitors of CAF-cancer interactions in anticancer therapy. On the other hand, if the therapeutic target were the CAF-cancer contacts and not CAFs themselves, the strategy might be safe because CAFs would not be depleted.

The use of CAF as a trans-shipment point for the delivery of genetic therapeutic constructs to cancer cells

Another feature of CAFs, important from the viewpoint of new therapeutic targets, is worth

noting: fibroblasts are more genetically stable than "true" cancer cells [21, 67]. They divide slowly and, accordingly, slowly mutate. Due to this, stromal therapeutic targets might be more stable compared to cancer cells with a permanently changing genetic structure.

Several strategies have now emerged to utilize therapeutic gene delivery to intentionally alter the CAFs. It has been shown that plasmid DNA can be delivered to, and expressed in, CAFs using lipid-based nanoparticles as carriers [68, 69]. The delivery of a gene that produced a soluble TNFa-related apoptosis inducing ligand (sTRAIL) to CAFs caused apoptosis in the tumor parenchyma, and ultimately tumor regression [69]. Similarly, several studies have shown that delivery to CAFs of genes encoding fusion proteins designed to be secreted and bound to soluble factors such as chemokines and cytokines in the tumor microenvironment can cause reduction of metastasis and ultimately improve survival in animal models.

Collectively, these results offer a proof of concept for the use of gene therapeutic constructs to modify CAFs for further transfer of therapeutics to cancer cells or their environment could be an effective strategy to treat cancers.

Conclusion

This review illustrates that cancer is no longer regarded just as a set of mutant and dysregulated epithelial cancer cells with their "driver" mutations. Instead, cancer and TME (stroma) cells jointly form an evolving, integrated, cooperative, and dynamic organ-like system. So, it becomes clear that in order to defeat cancer, we should abandon the attempts to treat by targeting the components of complex intracellular interactoms, and instead try to disrupt the system, as a whole, by destroying the interaction of its constituent parts. Further analysis of interactions and the development of systems for the delivery and expression of genes in CAF may lead to the emergence of a new approach that will significantly improve cancer therapy, especially in combination with checkpoint immunotherapy and more traditional methods such as chemo- and radiotherapy

Funding

REFERENCES

1. *Hanahan D, Weinberg RA*. The hallmarks of cancer. *Cell*. 2000;**100**(1):57–70.
2. *Hanahan D, Coussens LM*. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell*. 2012;**21**(3):309–22.
3. *Hanahan D, Weinberg RA*. Hallmarks of cancer: the next generation. *Cell*. 2011;**144**(5):646–74.
4. *Orimo A, Weinberg RA*. Stromal fibroblasts in cancer: a novel tumor-promoting cell type. *Cell Cycle*. 2006;**5**(15):1597–601.
5. *Gonda TA, Varro A, Wang TC, Tycko B*. Molecular biology of cancer-associated fibroblasts: can these cells be targeted in anti-cancer therapy? Semin Cell Dev Biol. *2010;21(1):2–10.*
6. *De Palma M, Hanahan D*. The biology of personalized cancer medicine: facing individual complexities underlying hallmark capabilities. *Mol Oncol*. 2012;**6**(2):111–27.
7. *Fouad YA, Aanei C*. Revisiting the hallmarks of cancer. *Am J Cancer Res*. 2017;**7**(5):1016–1036.

8. *Gascard P, Tlsty TD.* Carcinoma-associated fibroblasts: orchestrating the composition of malignancy. *Genes Dev.* 2016;**30**(9):1002–19.

9. *Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, Hu G, Sun Y.* New horizons in tumor microenvironment biology: challenges and opportunities. *BMC Med.* 2015;**13**:45.

10. *Gandellini P, Andriani F, Merlino G, D'Aiuto F, Roz L, Callari M.* Complexity in the tumour microenvironment: Cancer associated fibroblast gene expression patterns identify both common and unique features of tumour-stroma crosstalk across cancer types. *Semin Cancer Biol.* 2015;**35**:96–106.

11. *Stadler M, Walter S, Walzl A, Kramer N, Unger C, Scherzer M, Unterleuthner D, Hengstschläger M, Krupitza G, Dolznig H.* Increased complexity in carcinomas: Analyzing and modeling the interaction of human cancer cells with their microenvironment. *Semin Cancer Biol.* 2015;**35**:107–24.

12. *Zi F, He J, He D, Li Y, Yang L, Cai Z.* Fibroblast activation protein α in tumor microenvironment: recent progression and implications (review). *Mol Med Rep.* 2015;**11**(5):3203–11.

13. *Raffaghello L, Dazzi F.* Classification and biology of tumour associated stromal cells. *Immunol Lett.* 2015;**168**(2):175–82.

14. *Perrimon N, Pitsouli C, Shilo BZ.* Signaling mechanisms controlling cell fate and embryonic patterning. *Cold Spring Harb Perspect Biol.* 2012;**4**(8): a005975.

15. *Bizzarri M, Cucina A.* Tumor and the microenvironment: a chance to reframe the paradigm of carcinogenesis? Biomed Res Int. *2014;2014:934038.*

16. *Guo F, Wang Y, Liu J, Mok SC, Xue F, Zhang W.* CXCL12/CXCR4: a symbiotic bridge linking cancer cells and their stromal neighbors in oncogenic communication networks. *Oncogene.* 2016;**35**(7): 816–26.

17. *Sluka P, Davis ID.* Cell mates: paracrine and stromal targets for prostate cancer therapy. *Nat Rev Urol.* 2013;**10**(8):441–51.

18. *Bhome R, Bullock MD, Al Saihati HA, Goh RW, Primrose JN, Sayan AE, Mirnezami AH.* A top-down view of the tumor microenvironment: structure, cells and signaling. *Front Cell Dev Biol.* 2015;**3**:33.

19. *Weinberg RA.* Coming full circle-from endless complexity to simplicity and back again. *Cell.* 2014;**157**(1):267–71.

20. *Reina-Campos M, Moscat J, Diaz-Meco M.* Metabolism shapes the tumor microenvironment. *Curr Opin Cell Biol.* 2017;**48**:47–53.

21. *Bhome R, Al Saihati HA, Goh RW, Bullock MD, Primrose JN, Thomas GJ, Sayan AE, Mirnezami AH.* Translational aspects in targeting the stromal tumour microenvironment: from bench to bedside. *New Horiz Transl Med.* 2016;**3**(1):9–21.

22. *Kalluri R.* The biology and function of fibroblasts in cancer. *Nat Rev Cancer.* 2016;**16**(9):582–98.

23. *LeBleu VS, Kalluri R.* A peek into cancer-associated fibroblasts: origins, functions and translational impact. *Dis Model Mech.* 2018;**11**(4). pii: dmm029447.

24. *Valkenburg KC, de Groot AE, Pienta KJ.* Targeting the tumour stroma to improve cancer therapy. *Nat Rev Clin Oncol.* 2018;**15**(6):366–381.

25. *Munhoz RR, Postow MA.* Clinical development of pd-1 in advanced melanoma. *Cancer J.* 2018/ Feb;**24**(1):7–14.

26. *Postow M, Wolchok J.* Patient selection criteria and toxicities associated with checkpoint inhibitor immunotherapy. *UptoDate.* 2018.

27. *Sharma P, Allison JP.* The future of immune checkpoint therapy. *Science.* 2015;**348**(6230):56–61.

28. *Sharpe AH.* Introduction to checkpoint inhibitors and cancer immunotherapy. *Immunol Rev.* 2017;**276**(1):5–8.

29. *Wilson RAM, Evans TRJ, Fraser AR, Nibbs RJB.* Immune checkpoint inhibitors: new strategies to checkmate cancer. *Clin Exp Immunol.* 2018;**191**(2):133–148.

30. *Calabrese L, Velcheti V.* Checkpoint immunotherapy: good for cancer therapy, bad for rheumatic diseases. *Ann Rheum Dis.* 2017;**76**(1):1–3.

31. *Abdin SM, Zaher DM, Arafa EA, Omar HA.* Tackling cancer resistance by immunotherapy: updated clinical impact and safety of PD-1/PD-L1 inhibitors. *Cancers (Basel).* 2018;**10**(2). pii: E32.

32. *Postow MA, Hellmann MD.* Adverse events associated with immune checkpoint blockade. *N Engl J Med.* 2018;**378**(12):1165.

32. *Postow MA, Sidlow R, Hellmann MD.* Immune-Related Adverse Events Associated with Immune

Checkpoint Blockade. *N Engl J Med.* 2018; **378**(2):158–168.

33. *Kuhlmann L, Cummins E, Samudio I, Kislinger T.* Cell-surface proteomics for the identification of novel therapeutic targets in cancer. *Expert Rev Proteomics.* 2018;**15**(3):259–275.

34. *Kim JW, Cochran JR.* Targeting ligand-receptor interactions for development of cancer therapeutics. *Curr Opin Chem Biol.* 2017;**38**:62–69.

35. *Weikl T, Asfaw M, Krobath H, Rozycki B, Lipowsky R.* Adhesion of membranes via receptor–ligand complexes: Domain formation, binding cooperativity, and active processes. *Soft Matter.*2009; **5**(17): 3213–24.

36. *Cogdill AP, Andrews MC, Wargo JA.* Hallmarks of response to immune checkpoint blockade. *Br J Cancer.* 2017;**117**(1):1–7.

37. *Alexander W.* The checkpoint immunotherapy revolution: what started as a trickle has become a flood, despite some daunting adverse effects; new drugs, indications, and combinations continue to emerge. *P T.* 2016;**41**(3):185–91.

38. *Madden DL.* From a patient advocate's perspective: does cancer immunotherapy represent a paradigm Shift? *Curr Oncol Rep.* 2018; **20**(1): 8.

39. *Dempke WCM, Fenchel K, Uciechowski P, Dale SP.* Second- and third-generation drugs for immuno-oncology treatment-The more the better? *Eur J Cancer.* 2017;**74**:55–72.

40. *Fearon DT.* The carcinoma-associated fibroblast expressing fibroblast activation protein and escape from immune surveillance. *Cancer Immunol Res.* 2014;**2**(3):187–93.

41. *Day D, Monjazeb AM, Sharon E, Ivy SP, Rubin EH, Rosner GL, Butler MO.* From famine to feast: developing early-phase combination immunotherapy trials wisely. *Clin Cancer Res.* 2017;**23**(17):4980–4991.

42. *Santi A, Kugeratski FG, Zanivan S.* Cancer associated fibroblasts: the architects of stroma remodeling. *Proteomics.* 2018;**18**(5-6):e1700167.

43. *Marsh T, Pietras K, McAllister SS.* Fibroblasts as architects of cancer pathogenesis. *Biochim Biophys Acta.* 2013;**1832**(7):1070–8.

44. *Liao Z, Tan ZW, Zhu P, Tan NS.* Cancer-associated fibroblasts in tumor microenvironment - accom-plices in tumor malignancy. *Cell Immunol.* 2018. pii: S0008-8749(17)30222–8.

45. *Ziani L, Chouaib S, Thiery J.* Alteration of the antitumor immune response by cancer-associated fibroblasts. *Front Immunol.* 2018;**9**:414.

46. *Alkasalias T, Moyano-Galceran L, Arsenian-Henriksson M, Lehti K.* Fibroblasts in the tumor microenvironment: shield or spear? *Int J Mol Sci.* 2018;**19**(5). pii: E1532.

47. *De Wever O, Van Bockstal M, Mareel M, Hendrix A, Bracke M.* Carcinoma-associated fibroblasts provide operational flexibility in metastasis. *Semin Cancer Biol.* 2014;**25**:33–46.

48. *Heneberg P.* Paracrine tumor signaling induces transdifferentiation of surrounding fibroblasts. *Crit Rev Oncol Hematol.* 2016;**97**:303–11.

49. *Augsten M.* Cancer-associated fibroblasts as another polarized cell type of the tumor microenvironment. *Front Oncol.* 2014;**4**:62.

50. *Tao L, Huang G, Song H, Chen Y, Chen L.* Cancer associated fibroblasts: An essential role in the tumor microenvironment. *Oncol Lett.* 2017;**14**(3):2611–2620.

51. *Lamprecht S, Sigal-Batikoff I, Shany S, Abu-Freha N, Ling E, Delinasios GJ, Moyal-Atias K, Delinasios JG, Fich A.* Teaming up for trouble: cancer cells, transforming growth factor-β1 signaling and the epigenetic corruption of stromal naïve fibroblasts. *cancers (Basel).* 2018;**10**(3). pii: E61.

52. *Du H, Che G.* Genetic alterations and epigenetic alterations of cancer-associated fibroblasts. *Oncol Lett.* 2017;**13**(1):3–12.

53. *Belli C, Trapani D, Viale G, D'Amico P, Duso BA, Della Vigna P, Orsi F, Curigliano G.* Targeting the microenvironment in solid tumors. *Cancer Treat Rev.* 2018;**65**:22–32.

54. *Harper J, Sainson RC.* Regulation of the anti-tumour immune response by cancer-associated fibroblasts. *Semin Cancer Biol.* 2014;**25**:69–77.

55. *Lakins MA, Ghorani E, Munir H, Martins CP, Shields JD.* Cancer-associated fibroblasts induce antigen-specific deletion of CD8 (+) T Cells to protect tumour cells. *Nat Commun.* 2018;**9**(1):948.

56. *Rozali EN, Hato SV, Robinson BW, Lake RA, Lesterhuis WJ.* Programmed death ligand 2 in cancer-

induced immune suppression. *Clin Dev Immunol.* 2012;**2012**:656340.

57. *Nazareth MR, Broderick L, Simpson-Abelson MR, Kelleher RJ Jr, Yokota SJ, Bankert RB.* Characterization of human lung tumor-associated fibroblasts and their ability to modulate the activation of tumor-associated T cells. *J Immunol.* 2007;**178**(9): 5552–62.

58. *Jacobs J, Smits E, Lardon F, Pauwels P, Deschoolmeester V.* Immune checkpoint modulation in colorectal cancer: what's new and what to expect. *J Immunol Res.* 2015;**2015**:158038.

59. *Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K, Sahai E.* Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. *Nat Cell Biol.* 2007; **9**(12):1392–400.

60. *Semba S, Kodama Y, Ohnuma K, Mizuuchi E, Masuda R, Yashiro M, Hirakawa K, Yokozaki H.* Direct cancer-stromal interaction increases fibroblast proliferation and enhances invasive properties of scirrhous-type gastric carcinoma cells. *Br J Cancer.* 2009;**101**(8):1365–73.

61. *Choe C, Shin YS, Kim SH, Jeon MJ, Choi SJ, Lee J, Kim J.* Tumor-stromal interactions with direct cell contacts enhance motility of non-small cell lung cancer cells through the hedgehog signaling pathway. *Anticancer Res.* 2013;**33**(9):3715–23.

62. *He XJ, Tao HQ, Hu ZM, Ma YY, Xu J, Wang HJ, Xia YJ, Li L, Fei BY, Li YQ, Chen JZ.* Expression of galectin-1 in carcinoma-associated fibroblasts promotes gastric cancer cell invasion through upregulation of integrin β1. *Cancer Sci.* 2014;**105**(11):1402–10.

63. *Yamaguchi H, Sakai R.* Direct interaction between carcinoma cells and cancer associated fibroblasts for the regulation of cancer invasion. *Cancers (Basel).* 2015;**7**(4):2054–62.

64. *Labernadie A, Kato T, Brugués A, Serra-Picamal X, Derzsi S, Arwert E, Weston A, González-Tarragó V, Elosegui-Artola A, Albertazzi L, Alcaraz J, Roca-Cusachs P, Sahai E, Trepat X.* A mechanically active heterotypic E-cadherin/N-cadherin adhesion enables fibroblasts to drive cancer cell invasion. *Nat Cell Biol.* 2017;**19**(3):224–237.

65. *Theveneau E, Linker C.* Leaders in collective migration: are front cells really endowed with a particular set of skills? F1000Res. 2017;**6**:1899.

66. *Wang B.* Cancer cells exploit the Eph-ephrin system to promote invasion and metastasis: tales of unwitting partners. *Sci Signal.* 2011;**4**(175):pe28.

67. *Castells M, Thibault B, Delord JP, Couderc B.* Implication of tumor microenvironment in chemoresistance: tumor-associated stromal cells protect tumor cells from cell death. *Int J Mol Sci.* 2012;**13**(8):9545–71.

68. *Harrison EB, Azam SH, Pecot CV.* Targeting Accessories to the crime: nanoparticle nucleic acid delivery to the tumor microenvironment. *Front Pharmacol.* 2018;**9**:307.

69. *Miao L, Liu Q, Lin CM, Luo C, Wang Y, Liu L, Yin W, Hu S, Kim WY, Huang L.* Targeting tumor-associated fibroblasts for therapeutic delivery in desmoplastic tumors. *Cancer Res.* 2017;**77**(3): 719–731.

Взаємодії пухлина-строма як мішень для протипухлинної терапії

І. В. Алексеєнко, В. В. Плешкан, Є. Д. Свердлов

В ході еволюції пухлини ракові клітини використовують взаємодії пухлина-строма для реорганізації мікрооточення для досягнення максимальної стійкості пухлини. Успіх терапії з використанням імунних контрольних точок запропонував нову парадигму лікування раку: для перемоги раку, слід відмовитися від спроб його лікування, націлюючись лише на ракові, або тільки на стромальні клітини, або на компоненти складних внутрішньоклітинних взаємодій. Замість цього потрібно докладати зусиль для руйнування пухлини в цілому, розірвавши взаємодії між її частинами, зокрема, шляхом впливу на прямі контакти між власне раковими і стромальних клітинами пухлини. У цьому міні-огляді ми розглянемо можливість використання пухлина-асоційованих фібробластів (ОАФ) і їхню взаємодію з раковими клітинами як перспективний напрям терапії раку.

К л ю ч о в і с л о в а: рак, маркер, терапія, імунотерапія, строма, взаємодії

Взаимодействия опухоль-строма как мишень для противоопухолевой терапии

И. В. Алексеенко, В. В. Плешкан, Е. Д. Свердлов

В ходе эволюции опухоли раковые клетки используют взаимодействия опухоль-строма для реорганизации микроокружения с целью достижения максимальной устойчивости опухоли. Успех терапии с использованием иммунных контрольных точек породил новую парадигму лечения рака. Для того, чтобы победить рак, следует отказаться от попыток его лечения, нацеливаясь только на раковые, или только на стромальные клетки, или на компоненты сложных внутриклеточных взаимодействий. Вместо этого нужно предпринимать усилия для разрушения опухоли в целом, разорвав взаимодействия между ее частями, в частности, путем воздействия на прямые контакты между собственно раковыми и стромальными клетками опухоли. В этом мини-обзоре мы рассмотрим возможность использования опухоль-ассоциированных фибробластов (ОАФ) и их взаимодействий с раковыми клетками в качестве перспективного направления терапии рака.

Ключевые слова: рак, маркер, терапия, иммунотерапия, строма, взаимодействия