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TGFβ and activin A in the tumor microenvironment in colorectal cancer

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Abstract

Although overall survival in colorectal cancer (CRC) is increasing steadily due to progress in screening, therapeutic options and precise diagnostic tools remain scarce. As the understanding of CRC as a complex and multifactorial condition moves forward, the tumor microenvironment has come into focus as a source of diagnostic markers and potential therapeutic targets. The role of TGFβ in shifting the epithelial cancer compartment towards invasiveness and a pro-migratory phenotype via stromal signaling has been widely investigated. Accordingly, recent studies have proposed that CRC patients could be stratified into distinct subtypes and have identified one poor prognosis subset of CRC that is characterized by high stromal activity and elevated levels of TGFβ. The TGFβ superfamily member activin A is crucial for the pro-metastatic properties of the TGFβ pathway, yet it has been under-researched in CRC carcinogenesis. In this review, we will elucidate the signaling network and interdependency of both ligands in the context of the tumor microenvironment in CRC.

Keywords

TGFβ; activin A; tumor microenvironment; colorectal cancer

Introduction

Colorectal cancer (CRC) is among the deadliest cancers worldwide, approximately 50,000 annual cancer-associated deaths in the United States alone are attributed to CRC (1). Mortality is mainly due to metastatic disease, with 5-year survival rates as low as 14% in patients diagnosed at advanced stages (2). This circumstance has driven researchers to search for predictive biomarkers that indicate a tumor's propensity to metastasize and ideally to develop therapeutic strategies that specifically combat metastasis-prone tumors. Recent advances have been made in the understanding of colorectal cancer in its entirety, where the

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paradigm of carcinogenesis being only attributed to faulty epithelial cells is being questioned. Investigation of the tumor microenvironment (TME) has given us important insights into the complex metastasis-driven forces that do not originate from but are potentiated by the epithelial axis.

The TME is seen as the non-malignant entity which is comprised of cellular components (fibroblasts, mesenchymal stem cells, osteoblasts, fat cells, blood and immune cells) as well as the extracellular matrix (ECM) scaffold surrounding the cancerous cells (3, 4). The TME has gained a lot of attention as a provider of growth factors, cytokines and other pro-metastatic and anti-apoptotic molecules that orchestrate the malignant cell's ability to migrate. A body of evidence has emerged indicating that the interplay between the tumor and surrounding stroma is pivotal for a cancer to spread to distant sites, as first described by Paget's 'seed and soil theory' (5). Rather than regarding the TME as a stationary compartment, it should be considered as equally prone to changes like its surrounding cancer cells, and there is likely a parallel co-evolution of cancer cells and the TME (6). To co-opt and promote the pro-tumorigenic effects of the TME is necessary for cancer cells to progress, and the more stroma-rich a tumor is, the more aggressive the tumor becomes. Therefore, gene signatures and overexpressed proteins in the cancer tissue that are associated with stromal activity provide potential biomarkers that are indicative of patient prognosis and disease progression (7–11).

Transforming Growth Factor β (TGFβ) is major player in tumorigenic stroma-cancer interactions, and the tumor stroma is well known to be a rich source of TGFβ (12, 13). Given the abundance of the molecule and its pro-metastatic effects, TGFβ was intensively studied and targeted in many clinical trials, although with mixed results (14). The underlying problem of its underperformance as a drug target may be due to its highly context-dependent behavior as a tumor suppressor and promoter, and many aspects of its signaling network are still unclear. TGFβ superfamily member activin A is involved in and necessary for some TGFβ effects. As such, it has been shown that the invasive, pro-metastatic CRC phenotype induced by TGFβ is activin A-dependent. Furthermore, the fact that TGFβ and activin A expression correlates on the mRNA level in colorectal tumors and co-occurring mutations in their receptors are frequent, suggests a relationship between these signaling pathways (15). TGFβ and activin A are indispensable players in CRC metastasis, and assessment of the two molecules could therefore yield important prognostic information. Since they are not only structurally related, but their signaling pathways are entwined, TGFβ and activin A pathways should be viewed as a network, and the ligands should be assessed together (15, 16). Especially in settings of uncertainty of disease relapse (high-risk patients in stage II), TGFβ and activin A could serve as biomarkers to stratify patients into tailored chemotherapy regimens, and given their pro-metastatic effects, TGFβ and activin A could be exploited as dual drug targets in advanced CRC.

1. The bigger picture: The colorectal cancer microenvironment

The initiative to define a molecular signature for colorectal cancer was undertaken to provide prognostic and predictive information that goes beyond the classical TNM (primary **t**umor, lymph **n**ode, **m**etastasis) staging. However, aside from *KRAS* testing in metastatic

CRC (mCRC) and assessment for microsatellite instability (MSI), a diagnostic tool that encompasses mutational characterization of the epithelium has not been implemented in the clinic. The TME has become a focus of interest in CRC, as it is becoming evident that much of the prognostic information in fact lies in the composition of the environmental factors supporting cancer cell growth and metastases. For example, a high stromal fraction in tumor tissues is associated with poor prognosis and higher tumor staging (10, 17), as evidenced by a correlation between tumor-infiltrating immune cells and patient outcome (18).

The tumor microenvironment initially acts in a tumor-suppressive manner by default; however, at some point in carcinogenesis the TME fails this task and starts promoting pro-tumorigenic pathways. Furthermore, metastatic spread would not be possible without a favorable TME. Thus, researchers in the field have endeavored to identify factors that prompt the TME to switch to a tumor-promoting milieu with pro-metastatic functions. Recent studies indicate that TGFβ and activin A are intimately involved in this process (14, 19, 20).

2. TGFβ

TGFβ, a member of the TGFβ superfamily, plays a role in a spectrum of physiologic processes including growth, differentiation, and migration, but is also associated with fibrosis, immune suppression, and carcinogenesis (21–24). Other members of the TGFβ superfamily include activins, bone morphogenetic proteins (BMPs), nodals and growth and differentiation factors (GDFs) (25). In the healthy colon epithelium, TGFβ is crucial for homeostasis, as increasing gradients from crypt to surface control enterocyte growth. Furthermore, it is a mediator of intestinal immunity (26). There are three isoforms of TGFβ: TGFβ 1, TGFβ 2, TGFβ 3, with TGFβ 1 being the most prominent. TGFβ signals through receptors that are serine-threonine kinases that comprise a heterotetramer of two type I and two type II receptor subunits (TGFBRI and TGFBRII). A cellular response is elicited through binding of TGFβ ligand that initiates the type II receptor phosphorylation of the type I receptor, which in turn allows association of the receptor SMADs (R-SMADs) SMAD2 and SMAD3 (27). After dimerization with SMAD4, the complex translocates to the nucleus to initiate a transcriptional response. Other stimulated pathways that are not SMAD-dependent are considered non-canonical. This heterogeneous group includes PI3K/Akt, MAPK/Erk, WNT/β-catenin, Rho-like GTPases and JNK/p38 pathways (28, 29). TGFβ signaling is highly context-dependent, where the cellular response may be influenced by tissue type, concentration of ligands and mutations in pathway components (30).

Germline mutations in components of TGFβ superfamily signaling pathways have been linked to increased risk of developing CRC. For example, increased susceptibility to CRC is observed in individuals harboring germline mutations in the *BMPR1* and *SMAD4* genes, leading to a condition known as juvenile polyposis syndrome (JPS) (31). Patients with JPS develop juvenile hamartomatous lesions in the stomach, small intestine, and colon and have a 50% lifetime risk of developing GI cancer (32). Germline variations in the TGFβ receptors are also associated with higher risk of developing CRC, although the extent of the effect is likely modest (32).

TGFβ signaling has been described as tumor-suppressive in early stages of carcinogenesis, based on observations of their SMAD-dependent growth inhibition through p21 activation and induction of apoptosis (33–35). However, various reports of TGFβ in metastatic CRC add to the complexity of its framework. Inactivating mutations in pathway components such as SMAD4 are seen in 30% of cancers and are typically considered a late stage event associated with metastatic CRC (36). Loss of SMAD4 seems to be an almost exclusive event in microsatellite stable (MSS) cancers. In a study of protein expression of SMAD4 in sporadic colorectal neoplasia, only 4% of MSI carcinomas showed depletion of SMAD4 expression (37). Loss of canonical SMAD expression may be the main factor to co-opt the TGFβ signaling network and circumvent the tumor-suppressing effects, leaving only the non-canonical pro-tumorigenic and pro-metastatic functions, such as enhanced cell migration, cell growth and resistance to apoptosis (24, 34, 38, 39). Interestingly, in a study aiming at identifying high risk stage I and II patients, patients with high tumor stroma and loss of SMAD4 had the most unfavorable prognosis compared to stroma low and SMAD4 intact patients (40).

The non-canonical mitogenic MAPK/Erk and the survival-promoting PI3K/Akt pathway are two prominent targets of TGFβ, and are known to drive cancer cell malignancy (28). One of the main factors by which TGFβ facilitates metastatic spread is by the induction of EMT, a process by which cancer cells lose their epithelial polarity and tight junctions and express mesenchymal proteins such as vimentin. EMT is a hallmark of metastasis and is necessary for cancer cells to acquire mesenchymal characteristics that endow them with the ability to migrate and invade distant tissues (41). In a study of a 5-FU-resistant colorectal cancer cell line, drug resistance was associated with upregulation of EMT markers and a change in cellular morphology. As TGFβ is a prominent inducer of EMT, this suggests another mechanism as to how TGFβ can render CRC more aggressive (42).

2.1. TGFβ in the TME as master regulator of CRC malignancy—TGFβ has been extensively studied and is now recognized a main driver of metastasis in CRC. Although many of the mechanisms that alter TGFβ's behavior from tumor suppressor to promoter remain elusive, the field has reached consensus that TGFβ is predominantly over-represented in a late stage setting, and in combination with the TME, promotes disease progression and increases the likelihood of metastatic spread (43). TGFβ can be used as a prognostic biomarker in CRC, as it is indicative of survival and disease relapse (44). A recent meta-analysis concluded that CRC patients with either elevated TGFβ serum levels or high TGFβ protein or mRNA expression in the primary tumor have worse overall survival (with a hazard ratio (HR) of 1.68) compared to low TGFβ-expressing patients (44).This analysis strongly suggests that TGFβ is pivotal for cancer progression. However, mutations of TGFβ pathway components are frequent in CRC, such as inactivating mutations in TGFBRII (45). What may seem paradoxical at first can be explained by the notion that TGFβ exerts its pro-metastatic functions through effects on the TME rather than the epithelium. Calon et al. showed that epithelial cancer cells are able to initiate metastasis through a stromal TGFβ-guided response (12). According to their study, TGFβ response signatures (TBRS) in TME cell types (T-cells, macrophages, and fibroblasts) were predictors of disease relapse in stage I-III patients. This group also developed an *in vivo* model to

investigate the metastatic action of TGFβ in the stroma. TGFβ signaling was inhibited in a CRC epithelial cell line by mutating TGFβ receptor 2. TGFβ ligand was then overexpressed in these cells and inoculated in the caecum of nude mice. Compared to mice with non-overexpressing tumors, mice with TGFβ-overexpressing tumors developed significantly more metastases. Thus, this study points to the importance of TGFβ signaling in the stroma specifically to promote metastasis and underscores the need for further study of the TME in CRC progression (12).

2.2. The immune landscape and its modulation by TGFβ—Suppressing antitumor immunity is a hallmark for cancer progression and is controlled by TGFβ on many levels. The cytokine represses cytolytic activity of CD8+ cytotoxic T-lymphocytes (CTL) by inhibiting granzyme B, perforin and FAS-L (46), inhibits T-cell proliferation, reduces antigen spreading (47), reduces T-cell activation, and mediates Treg induction and activity (48–50). The interplay between TGFβ and the adaptive immunity in CRC has been demonstrated in a study of an *in vivo* model of the metastatic consensus molecular subtype 4 (CMS4) which is defined by a TGFβ-rich stroma and first described by Guinney et al. (51). In this study, upon depletion of CD8+ CTL and CD4+ T-helper cells, metastatic tumors were no longer responsive to TGFBRI inhibitor Galunisertib, suggesting that the anti-tumor effect of TGFβ pathway inhibition is dependent on a functional adaptive immune system. Furthermore, mice receiving Galunisertib in combination with anti-PD-L1 antibodies had more remissions and longer disease-free survival than mice treated with Galunisertib alone. This finding might be crucial for combatting metastasis, as dual targeting of TGFβ and PD-1/PD-L1 might have synergistic effects (51, 52). The authors further demonstrated that in an MSS patient cohort, TGFβ1, 2 and 3 expression negatively correlated with the ratio of TH1/TH-naïve cells, implying that TGFβ might suppress T-cell maturation (51, 53). Another report showed that dysregulation of the ECM through TGFβ overexpression in ‘immunogenically hot’ tumors predicted failure of treatment with PD-1 inhibitors, again implying TGFβ to be a pivotal modulator of the adaptive immunity, and substantiating the rationale of dual targeting of TGFβ and PD-1/PD-L1 (54).

The effects by which TGFβ regulates the innate immunity are just as meaningful to promote cancer progression (55). As such, TGFβ acts as a chemoattractant for neutrophils and polarizes them to the pro-tumorigenic N2-phenotype (56). Subsequently, N2 TANs are able to undermine the antitumor immunity (57). Furthermore, cancer-associated fibroblast (CAFs), which are primarily induced by TGFβ, have been shown to shift macrophage populations in CRC towards M2 (58). Taken together, a main mode of action for TGFβ is fine-tuning the tumor-promoting effects of the immune landscape in colorectal cancer.

2.3. Desmoplastic tumor stroma and TGFβ—In wound healing, epithelial cells are physiologically able to migrate through the tissue by undergoing EMT. That, along with activated contractile myofibroblasts, is necessary for wound closure (59). Desmoplasia, a phenomenon that creates a rigid microenvironment that ‘forces’ tumor cells to undergo EMT and metastasize, is sustained by the pro-fibrotic effects of TGFβ (60). As such, TGFβ activates EMT in tumor cells, induces α-SMA expression in fibroblasts to transform them to the CAF phenotype, and promotes deposition of ECM components, ultimately leading to

increased tissue rigidity through positive feedback loops (61, 62). As tumors progress, tissue density increases, and cancer cells are confronted with remarkable mechanical forces exerted by the contractile abilities of TGFβ-activated CAFs. A stiffening microenvironment further promotes CAF differentiation (63, 64) and increases TGFβ secretion (65), creating a vicious cycle and a self-sustained imbalance that ultimately increases the cancer cell's propensity to metastasize.

3. The role of activin A in the TGFβ signaling network

Activin A, a member of the TGFβ superfamily, was originally described as a multifunctional protein in embryonic development as well as gonadal and pituitary physiology (66). The cytokine has been studied in the context of esophageal (67), skin (68), ovarian (69), lung (70), breast (71, 72), pancreatic (73) and colorectal cancer (74, 75) and may be critical in cancer cachexia (76). Similar to the TGFβ pathway, activin A binds to serine/threonine kinase receptors. Three type I receptors (ACVRIA, ACVRAIB, ACVRIC) and two type II receptors (ACVRIIA, ACVRIIB) exist (77). Dimerization of a type I and type II receptor after ligand binding allows phosphorylation of the type I receptor and leads to activation of the canonical SMAD2/3 cascade to elicit a transcriptional response (78).

In the context of CRC, inactivating mutations of ACVRIIA alongside TGFBRII mutations are very common in patients with MSI CRC. Microsatellite instability causes frequent frameshift mutations within the polyadenine tracts of exon 10 in ACVRIIA and exon 3 in TGFBRII (79, 80). It has been reported that stage III and high-risk stage II patients with MSI receiving adjuvant chemotherapy harboring defective TGFβ receptors have a better 5-year disease-free survival compared to non-TGFBRII mutated MSI patients (81). However, this survival advantage has not been shown for ACVRII (82, 83). One study observed mutations in ACVRII to be associated with metastasis and decreased survival (84). Conflicting with these reports, it has consequently been shown that overexpression of activin A in CRC tissues is associated with stage IV tumors and indicates lower overall survival (74, 85), and serum activin A levels positively correlate with disease stage (86). These discrepancies can be explained by the fact activin A signaling, similar to TGFβ signaling, is context dependent. As with TGFβ, activin A plays a much larger role in the TME than in the epithelial cells. Therefore, assessing the mutational status of activin pathway components in the epithelial compartment might not yield qualitative prognostic information.

The activation of canonical SMAD2/3 and subsequent dimerization with SMAD4 is the core similarity between activin A and TGFβ signaling pathways (87). Despite distinct receptors, activin A and TGFβ pathways in CRC are often seen as redundant due to shared canonical SMAD signaling. However, it has been reported that canonical as well as non-canonical signaling patterns are divergent. p21, a primary transcriptional target of activated SMADs, carries out different cellular effects depending on whether the activin A or TGFβ signaling pathway is activated. For example, activin A-associated p21 signaling induces apoptosis, whereas TGFβ promotes growth suppression (34).

Non-canonical pathways associated with activin A and TGFβ are also distinct. It has been shown that activin A induces a downstream PI3K/Akt response, and TGFβ engages the MAP/Erk pathway to enable EMT (88). Interestingly, activated Erk is able to phosphorylate

SMAD2 and 3 on alternative sites, which impedes canonical, tumor-suppressive transcriptional activity (89). As concerning non-canonical activin A signaling, the beforementioned loss of SMAD4 causes upregulation of Akt, and in the clinical context, patients with SMAD4 mutations are more likely to show increased protein expression of phosphorylated Akt, which in turn predicts poor prognosis (90).

Activin A is increasingly recognized as a player in the metastatic process in CRC, as it carries out many of the malignant effects of TGFβ. As such, in a study of a CRC cell line, the pro-metastatic phenotype of TGFβ-treated cells was found to be activin A-dependent (15). The authors of the same study also report that activin A and TGFβ should be assessed together, as combined activin A and TGFβ protein expression scores in stage II patients yield a better prognostic information than either ligand alone (15). Acknowledging the differences in activin A and TGFβ while recognizing them as functionally intertwined is an important milestone and justifies continued investigation.

3.1. Activin A in the TME—Not only is activin A an important component in TGFβ signaling, the cytokine itself has many effects on cells of the TME to promote metastasis. Activin A is one of the first responders in wound healing, elevated mRNA expression of *INHBA*, the gene encoding for the β A subunit of activin A, is observed within 24 hours of wound infliction (91). However, perpetual activin A activity in tumors might add to the desmoplastic process, as overexpression of activin A is associated with excessive scarring and fibrosis (92). Strengthening the concept of activin A as a mediator in desmoplasia, CAFs secrete activin A in contrast to non-activated fibroblasts (93, 94), and activin A release can be potentiated by increased tissue stiffness (95). Strikingly, fibroblasts are a richer source of activin A than epithelial cells, and treatment with TGFβ leads to increased activin A release in epithelial and stromal cells, again underscoring a close reciprocal relationship of both ligands in the desmoplastic process (15).

The upregulation of MMPs by activin A provides another mechanistic insight into its function in facilitating metastasis. Induction of MMP-7 by activin A is necessary for dissolving the basement membrane and other components of the ECM to enable metastatic spread (96). Early increase of activin A in wounds and inflammatory processes establishes a logical link to its capacity to regulate inflammation and the innate immunity (97). This notion is corroborated by a study of LPS-stimulated mice, where the cytokines TNF-α and IL-1β were decreased after treatment with activin A antagonist follistatin (98).

It is still under debate whether activin A is considered a pro- or anti-inflammatory cytokine. The answer, as in many cases, may lie in the conditions where the molecule is active. Activin A was shown to have suppressive effects on pro-inflammatory IL-6 in a study of rheumatoid arthritis (99). Interestingly IL-6 regulation in amnion cells seems to be activin A dose-dependent; low quantities of activin A lead to a decrease in IL-6, whereas high amounts lead to a IL-6 increase, highlighting its complex role in coordinating inflammatory processes (100). *INHBA* is overexpressed in patients with inflammatory bowel diseases (101). The role of activin A in CRC-associated inflammation remains elusive. It has been reported that activin A is able to induce both the inflammatory M1 and the pro-tumorigenic

M2 phenotype in macrophages (102, 103). Activin A modulates neutrophil function (104), but further studies are needed to investigate whether activin A influences their polarization.

Activin A has been demonstrated to influence the function of T-cells, as such it is able to induce Treg cells, which suppress antitumor immunity (105–107). Intriguingly, activin A is able to convert CD4+CD25- T-cells into iTreg that express FOXP3+ in a TGFβ-dependent manner (108), again hinting at a close relationship between activin A and TGFβ signaling. In line with these findings, a recent report showed that Treg induced by TGFβ increases mRNA expression of activin-receptor 1 (ACVRI) and activin A ligand (50). This suggests that TGFβ and activin A synergize to promote Treg activity and act in tandem to suppress anti-cancer immune responses.

3.2. TGFβ and activin A as drug targets—The context-dependent behavior and the various physiologic functions exerted by activin A and TGFβ yield challenges to targeting the pathways for cancer treatment. Nevertheless, the prominence of both molecules in advanced CRC makes them attractive targets in a late stage setting, or in situations with high likelihood of metastatic spread. However, patients need to be carefully selected for treatment to specifically combat pro-metastatic effects and ensure tumor-suppressive functions are not abolished. One approach could be to assess for activin A and TGFβ in tumor samples with emphasis on the stromal levels of the ligands. Another tool could be to use established fibroblast and T-cell response signatures (F-TBRS, T-TBRS) of TGFβ (12, 109) and activin A to help identify patients who would benefit the most from anti-activin A or anti-TGFβ treatment. Since the TGFβ and activin A signaling networks are intertwined and respective receptors are structurally related, small molecule inhibitors that target both activin A and TGFβ receptors could be the most effective option (110). Thus far, targeting the TGFβ pathway has been conducted by four different approaches: small molecule inhibitors, neutralizing antibodies, fusion proteins, and vaccines, whereas small molecule inhibitors and one ligand trap are the most promising tools for targeting activin A (Table 1). Current clinical trials are exploring the synergistic effects of drugs targeting the PD-L1 and TGFβ pathway, as the combination has been shown to be efficacious in various preclinical modalities of advanced cancer (51, 111). Two ongoing clinical trials are targeting TGFβ and PD-L1/PD-1 with separate drugs, and one utilizes a combined PD-L1/TGFβ ligand trap (https://clinicaltrials.gov : NCT02734160; NCT02423343; NCT03620201). As activin A has a similar potency in suppressing antitumor immunity as TGFβ, future studies will show whether combined inhibition of immune checkpoints and activin A can be effective in selected patient populations (68).

Conclusion

Since the discovery of the multistep adenoma-to-carcinoma sequence, it took many years to uncover that cancer cells cooperate with and are controlled by various cues of the TME. This paradigm shift helped us understand the metastasis-driving forces outside the epithelium and has led to the discovery of predictive and prognostic biomarkers, as well as potential therapeutic targets. For successfully combatting CRC, combination therapies that entail specific targets for cancer cells as well as the TME will be the most efficient solution. Investigating activin A and TGFβ, key players in the TME, has provided insights into how

these molecules are able to orchestrate the pro-metastatic actions in the TME. Their tumor-promoting behavior in late stage cancers is an excellent example how progressing cancer cells co-opt a signaling network that is tumor-suppressive by nature. Activin A and TGFβ could serve as biomarkers for risk stratification in early and advanced tumor stages (high - risk stage II and stage III) and may be promising drug targets in patients where cancers have already metastasized. Future research will elucidate the precise mechanisms by which activin A and TGFβ influence functions of all cell types in the TME and will help us understand the crosstalk and synergism of both molecules in CRC. Furthermore, high throughput screening of colorectal tumors will aid to distinguish between a specific subtype of CRC with an activin A/TGFβ enriched TME by identifying response signatures of both molecules. Taken together, activin A and TGFβ are promising markers and targets in CRC, and their further investigation will help us develop necessary individual tailored therapies.

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Abbreviations

CAF	cancer-associated fibroblast
CMS	consensus molecular subtype
CRC	colorectal cancer
CTL	cytotoxic T-lymphocyte
ECM	extracellular matrix
EMT	epithelial to mesenchymal transition
IL	interleukin
mCRC	metastatic colorectal cancer
MMP	matrix metalloprotease
MSI	microsatellite instability
MSS	microsatellite stable
PD1	programmed cell death protein 1
PD-L1	programmed death-ligand 1
TAM	tumor-associated macrophage
TAN	tumor-associated neutrophil

TBRS	TGFβ response signature
TME	tumor microenvironment
TNF-α	tumor necrosis factor α
VEGF	Treg, regulatory T cell, vascular endothelial growth factor
5-FU	fluorouracil

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Highlights

- The tumor microenvironment is a pivotal driver of colorectal cancer metastasis
- TGFβ and activin A are the main determinants of a stroma-rich colorectal cancer subtype with poor prognosis
- Assessing activin A and TGFβ in tumors can identify a metastasis-prone subset of colorectal cancer and guide individual-tailored therapies

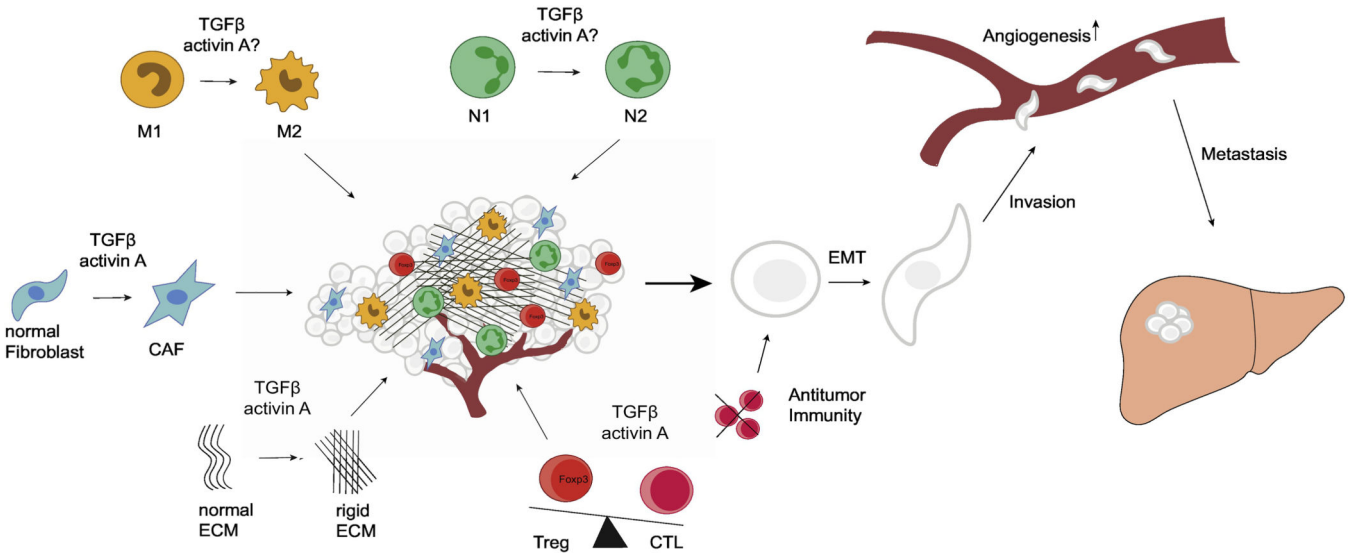

Figure 1:

The metastasis-promoting effects on the tumor microenvironment by activin A and TGFβ. Whereas TGFβ has been shown to shift neutrophil and macrophage populations towards N2/M2, the effects of activin A on those leukocytes are not as clear. Both molecules can induce the CAF-phenotype in fibroblasts, and reciprocally participate in the desmoplastic process in tumors. Desmoplasia is potentiated by a rigid ECM and the abundance of CAFs. Activin A and TGFβ are efficient suppressors of antitumor immunity and are able to induce immunosuppressive regulatory T-cells. The effects of activin A and TGFβ on the tumor microenvironment lead to increased cancer cells migration through induction of EMT, as well as angiogenesis and cancer cell invasiveness to ultimately accelerate the metastatic process.

(*N1* classically activated neutrophils, *N2* pro-tumor neutrophils, *M1* classically activated macrophages, *M2* pro-tumor macrophages, *CAF* cancer-associated fibroblasts *CTL* cytotoxic T-cells, *Treg* regulatory T-cells, *EMT* epithelial-to-mesenchymal transition)

Table 1:

Therapeutic approaches to inhibit components of the activin A and TGFβ pathways in solid tumors. (ALK-1 activin receptor-like kinase, TGFBRI TGFβ receptor 1, ACVRI activin receptor 1)

Name of drug	Target	Clinical phase	Type of cancer	Reference	Trial registration number	Status/Main outcome
TGFβ targeting agents						
Small molecule inhibitors						
PF-03446962	ALK-1	Phase I	Hepatocellular carcinoma (HCC)	(112)	NCT00557856	Completed/No complete or partial responses. 50% had stable disease
TEW-7197 (Vactosertib)	TGFBRI	Phase I	Advanced stage solid tumors	(113)	NCT02160106	Completed/Well tolerated, Vactosertib showed increased efficacy in patients with high fibroblast TGFβ response signature (F-TBRS)
LY3200882	TGFBRI	Phase I	Solid tumors		NCT02937272	recruiting
LY2157299 (Galunisertib)	TGFBRI	Phase I/II	Metastatic colorectal cancer (mCRC)		NCT03470350	recruiting
		Phase II	Metastatic Prostate cancer		NCT02452008	recruiting
Ligand traps						
AVID200	TGFβ 1, TGFβ 3	Phase I	Metastatic solid tumors		NCT03834662	recruiting
Vaccines						
bi-shRNAi^{furin}/ GMCSF DNA/ Autologous Tumor Cell Vaccine (FANG)	short hairpin RNAi (bi-shRNAi) targeting furin convert se to block TGFβ 1 and TGFβ 2 activation	Phase II	Metastatic colorectal cancer (mCRC)		NCT01505166	terminated
Antibodies						
GC1008 (Fresolimumab)	TGFBRI	Phase II	Metastatic breast cancer	(114)	NCT01401062	Completed/ Fresolimumab + radiation therapy, patients receiving 10mg/kg had significantly higher median overall survival than patients with 1mg/kg
Activin A targeting Agents						
Small molecule inhibitors						
ACE-011 (Sotatercept)	ACVRII	Phase II	Non-small cell lung cancer (NSCLC)	(115)	NCT01284348	terminated
		Phase II	Metastatic breast cancer		NCT00931606	terminated
Ligand traps						
STM 434	receptor-Fc fusion protein	Phase I	Advanced solid tumors	(116)	NCT02262455	Completed/53.5% had stable disease

Name of drug	Target	Clinical phase	Type of cancer	Reference	Trial registration number	Status/Main outcome
	against activin A					