Revealing neuropilin expression patterns in pancreatic cancer: From single-cell to therapeutic opportunities (Review)

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Abstract. Pancreatic cancer, one of the most fatal types of human cancers, includes several non-epithelial and stromal components, such as activated fibroblasts, vascular cells, neural cells and immune cells, that are involved in different cancers. Vascular endothelial cell growth factor 165 receptors 1 [neuropilin-1 (*NRP-1*)] and 2 (*NRP-2*) play a role in the biological behaviors of pancreatic cancer and may appear as potential therapeutic targets. The NRP family of proteins serve as co-receptors for vascular endothelial growth factor, transforming growth factor β, hepatocyte growth factor, fibroblast growth factor, semaphorin 3, epidermal growth factor, insulin-like growth factor and platelet-derived growth factor. Investigations of mechanisms that involve the NRP family of proteins may help develop novel approaches for overcoming therapy resistance in pancreatic cancer. The present review aimed to provide an in-depth exploration of the multifaceted roles of the NRP family of proteins in pancreatic cancer, including recent findings from single-cell analysis conducted within the context of pancreatic adenocarcinoma, which revealed the intricate involvement of NRP proteins at the cellular level. Through these efforts, the present study endeavored to further reveal their relationships with different biological processes and their potential as therapeutic targets in various treatment modalities, offering novel perspectives and directions for the treatment of pancreatic cancer.

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Contents

1. Introduction

Vascular endothelial cell growth factor 165 receptor 1 [neuropilin-1 (*NRP-1*)] is a protein-coding gene on human chromosome 10p11.22 that codes the NRP-1 protein from 923 amino acids (103 kDa), a cell surface receptor that contains protein domains that allow their participation in different types of signaling pathways that control cell migration (1). A family gene, *NRP-2* (human chromosome 2q33.3), encoding a novel member of the family protein neuropilin-2 (NRP-2) that contains 931 amino acids with 104 kDa, was identified as a high-affinity receptor for the Semaphorins (2). Previous studies revealed that NRP family proteins exert multiple functions as co-receptors for vascular endothelial growth factor (VEGF) (3), transforming growth factor beta (TGF-β) (4), hepatocyte growth factor (5), fibroblast growth factor (FGF) (6), and Semaphorin 3 (SEMA3) (7). NRP family proteins are involved in the interaction with multiple ligand receptors, thus NRP family proteins may be involved in cancer occurrence and development and might serve as therapeutic targets for gastric cancer (8), glioma (9), endometrial cancer (10), bladder cancer (11), thyroid cancer (12), breast cancer (13), gallbladder cancer (14), colorectal cancer (15), and pancreatic adenocarcinoma (PDAC) (16). Moreover, NRP signaling has been associated with several biological processes, including pro-tumorigenic cell proliferation, invasion, metastasis, and tumor growth in PDAC (17). NRP signaling can provide resistance to chemotherapeutic reagent exposure in clinical settings

by imitating the therapy-resistant cancer stem-cell properties (18). Recent advances in single-cell analysis (SCA) have revealed multiple functional roles of cancer-related cellular proteins (19), but the roles of NRP remain poorly understood. Here, we discuss recent advances in NRP biology in PDAC based on the SCA-based precision study.

2. NRP signaling

NRP-1 contains a large N-terminal extracellular domain, including complement-binding, coagulation factors V/VIII (CF V/VIII), and meprin domains. The two NRP-1 (complement-binding) CUB domains and the amino-terminal CF V/VIII domain are crucial for SEMA3A binding. The amino-terminal NRP-1 CF V/VIII domain remains the only required for binding to VEGF-165. Therefore, NRP-1 exerts its biological functions by binding with Semaphorin ligands (20). A previous study revealed that SEMA3A can inhibit axonal growth and induce neuronal apoptosis after binding to NRP-1, with the membrane-proximal meprin, A5/NRP1, protein tyrosine-phosphatase μ (MAM) domain of NRP-1. NRP-1 is involved in regulating cell survival by mediating the effects of its ligands, such as VEGF. NRP-1 promotes survival in cancer cells by activating signaling pathways, such as the PI3K/AKT pathway (21). The activation of these pathways helps in tumor progression and therapy resistance. Additionally, NRP-1 plays an essential role in cell migration by mediating the effects of Semaphorins and VEGF. NRP-1 can enhance the invasive and migratory capabilities of cancer cells. NRP-1, by interacting with its ligands, can activate downstream signaling pathways, such as Src kinases, which modulate cytoskeletal dynamics and cell adhesion, thereby promoting cell migration and invasion (22). This causes tumor metastasis, where cancer cells spread to other body parts. These results indicate that the meprin domain is involved in forming a higher-order receptor complex. NRP may play a key role in cell-to-cell interaction via their responses to ligands (Fig. 1) (23). Moreover, a recent report indicated the involvement of the NRP-1 signal in the symmetric cell division to expand breast cancer stem-like cells (24). NRP-1 has been overexpressed in various cancer types, including lung, breast, pancreatic, and prostate cancers. NRP-1 affects cell survival, migration, and attraction by binding to ligands and various co-receptors and may serve as a cancer biomarker of refractory tumors (25).

NRP-2 is characterized by a transmembrane protein that binds to the SEMA domain, immunoglobulin domain (Ig), Semaphorin 3C (SEMA3C), and Semaphorin 3F (SEMA3F) (26), and NRP-2 interacts with VEGF (27). NRP-1 binds with high affinity to the three structurally related Semaphorins, such as SEMA3, SEMAE, and SEMA4, whereas NRP-2 shows high-affinity binding to SEMAE and SEMA4, but not SEMA3 (2). NRP-2 is involved in cardiovascular development, axon guidance, and tumorigenesis (28,29).

Neuropilins (NRPs) are transmembrane glycoproteins that act as co-receptors for a variety of ligands, including vascular endothelial growth factor (VEGF), semaphorins (SEMA), and transforming growth factor-beta (TGF-β). These ligands bind to NRPs, which then interact with and enhance the signaling of their respective receptors, such as VEGF receptor (VEGFR) and TGF-β receptor (TGFBR). VEGF is a key regulator of angiogenesis, and its binding to NRP-1 enhances VEGFR-2 signaling, leading to endothelial cell proliferation and migration. SEMA3s are involved in axon guidance and immune regulation, and their binding to NRP-1 and NRP-2 can activate downstream signaling pathways such as RhoA/ROCK and PI3K/Akt. TGF-β is a multifunctional cytokine that plays a critical role in cell growth, differentiation, and immune regulation. Its binding to NRP-1 enhances TGFBR signaling, leading to downstream activation of Smad2/3 and other signaling pathways. NRPs have been reported to interact with various signaling pathways, including TGF-β, PDGF, FGF, c-Met, and others (Fig. 1). Despite some controversy surrounding these interactions, current knowledge suggests that NRP-1 has been involved in cancer stem-cell maintenance and progression through the Wnt/β-catenin signaling pathway (30) whereas NRP-2 has been associated with lymphangiogenesis and lymphatic metastasis in certain cancer types (31).

3. Intractable PDAC

Pancreatic cancer, also known as PDAC, is one of the most aggressive cancers globally. A PDAC diagnosis carries a 5-year survival rate of <10% (32,33). PDAC's clinical aggressiveness has been attributed to the i) lack of PDAC-specific symptoms (rendering early-stage detection difficult) (34-36), ii) early metastases (typically spreading to marginal tissues and distant organs, including the liver) (34,35), and iii) chemo- and radiotherapy resistance (34,37). Importantly, many other factors, such as topographical, vascular, and ductal pancreatic anatomy (38), and the complex involvement of stromal components of PDAC (39), may be involved in high disease recurrence rates.

Studies of six cohorts, comprising 136,000 cells from 71 cases with PDAC, indicated that PDAC contains various cells, including cancer-associated fibroblasts (CAFs) to understand the complexity of PDAC's cellular components. CAFs facilitate cell-to-cell communication and are involved in PDAC spread and therapeutic resistance (40,41). They were classified into several subpopulations, including inflammatory CAF (iCAF), myofibroblast CAF (myCAF), and antigen-presenting CAF (apCAF), based on gene expression (41). Diverse CAF subpopulations were reported for nine cancer types (42). PDAC that is characterized by iCAFs, which express interleukin 6 (IL6), collagen type XIV alpha 1 chain (COL14A1), lymphocyte antigen 6 complex locus C1 (LY6C), etc., was classified as 'classic-type' with a strong inflammatory profile (41). PDAC that is characterized by myCAFs, which express actin alpha 2, smooth muscle (ACTA2/aSMA), transgelin (TAGLN), thrombospondin 2 (THBS2), leucine-rich repeat containing 15 (LRRC15), etc., was considered as 'basal-type' with a strong myofibroblast profile (41). ApCAFs, which represent a distinct subset of CAFs expressing major histocompatibility complex class II (MHC II) and CD74, possess antigen-presenting capabilities. However, they notably lack the expression of co-stimulatory molecules, such as CD40, CD80, and CD86, resulting in the inability to initiate the typical activation response in $CD4^+$ T cells. The specific role of apCAFs remains unclear, but a widely accepted hypothesis indicates that they might attract $CD4^+$ T cells by expressing MHCII and subsequently interfering with their normal functionality.

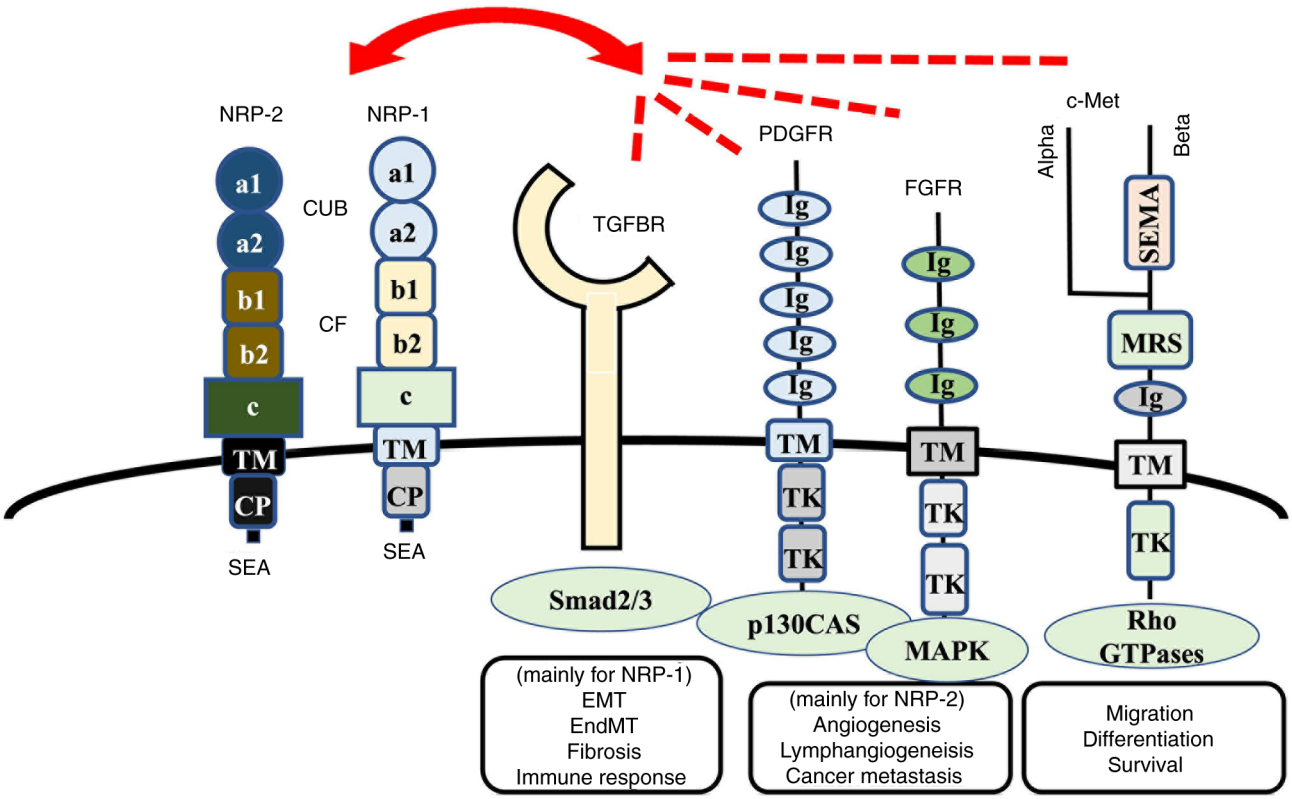

Figure 1. NRP-1 and NRP-2 and their related receptors. NRP-1 is a cell membrane-bound receptor that consists of three extracellular domains: i) a1/a2 domain, homologous to the complement proteins C1r/C1s, Uegf and Bmp-1 (referred to as the CUB domain); ii) b1/b2 domain, which is homologous to the coagulation factors V and VIII; and iii) c domain, which is homologous to meprin, A5 protein and protein tyrosine phosphatase μ, as well as TM and CP. NRP-1 contains an SEA sequence in the C-terminus that represents a consensus binding motif for proteins that contain the PDZ (PSD-95, Dlg, ZO-1) domain, such as synectin, which can act as the docking site for interacting partners. The homologies between NRP-1 and NRP-2 are 55% (in the a1/a2 domain), 48% (in the b1/b2 domain), 35% (in the c domain) and 49% (in the CP region) (75). TGF binds to cell membrane-bound serine/threonine kinase receptors that belong to the TGF-β receptor family. PDGFRs consist of extracellular five Ig-like domains and intracellular tyrosine kinase domains, whereas FGFRs consist of extracellular three Ig-like domains and intracellular tyrosine kinase domains. NRP-1 and NRP-2 interact with those receptors and modulate the biological function of cancer cells, vessel and lymphatic endothelial cells, fibroblasts and immune cells, which are components of architectures in tumor microenvironments. NRP, Neuropilin; TM, transmembrane domain; CP, cytoplasmic region; PDGFRs, platelet-derived growth factor receptors; FGFRs, fibroblast growth factor receptors; SEMA, Semaphorins; MRS, Met-related sequence; TK, tyrosine kinase; TGFBR, TGF-β receptor; EMT, epithelial-to-mesenchymal transition; EndMT, endothelial-to-mesenchymal transition; SEA, cytoplasmic domain.

This interference causes $CD4^+$ T cell inactivation or differentiation into regulatory T cells, thereby potentially contributing to the development of an immunosuppressive tumor microenvironment (43,44). Inter-cellular communication via ligands and their receptors indicated that sonic hedgehog (Shh)-mediated signals in CAFs suppressed cancer cell proliferation and progression in a PDAC model (45).

4. NRP expression in single PDAC cells

Few reports have focused on NRP expression at the single-cell level in PDAC, thus we used a published single-cell database (https://zenodo.org/record/6024273#.Y7T3tNXP1D8) to examine 136,000 cells from 71 patients with PDAC (41). We revealed various NRP expressions in human PDAC cells, which expressed both NRP-1 and NRP-2. In contrast, ductal cell type 1, another cell cluster, was positive for NRP-1 but not NRP-2. Thus, NRP-1 and NRP-2 appear to allow ductal cells in the pancreas to fulfill different functions. NRP-1 expression, in stellate cells, was higher than NRP-2. Fibroblasts, macrophages, and endothelial cells expressed substantial amounts of both NRP-1 and NRP-2. Endocrine cells featured very few NRP-1 or NRP-2 expressions, indicating that cases with aggressive phenotypes demonstrate fewer endocrine cells. MyCAF cells tend to express both NRP-1 and NRP-2 at high levels, while iCAF cells express only NRP-1 at high levels. Additionally, SEMA3A expression was similar in myCAF and iCAF, but the number of FGF1-expressing cells appeared slightly higher in myCAF. Targeting NRP signaling may represent a potential PDAC therapy approach, considering the high expression of NRP-1 and NRP-2 in ductal cells and fibroblasts.

Therapeutic targeting of NRP-1-positive cells in PDAC can regulate endothelial-to-mesenchymal transition (EndMT), which is an important source of fibroblasts in pathological disorders, thereby reducing tumor fibrosis and PDAC progression (46). The tumor-penetrating peptide was reported via a transcytosis transport pathway that is regulated by NRP-1. This system enhances the transcytosis of silicasome-based chemotherapy for PDAC in NRP-1-positive cells (47). Chimeric antigen receptor T cell (CAR-T) immunotherapy allows T cells to recognize an antigen and attach to antigen-positive cells, thus CAR-T targeting NRPs might be a potential PDAC therapy (8).

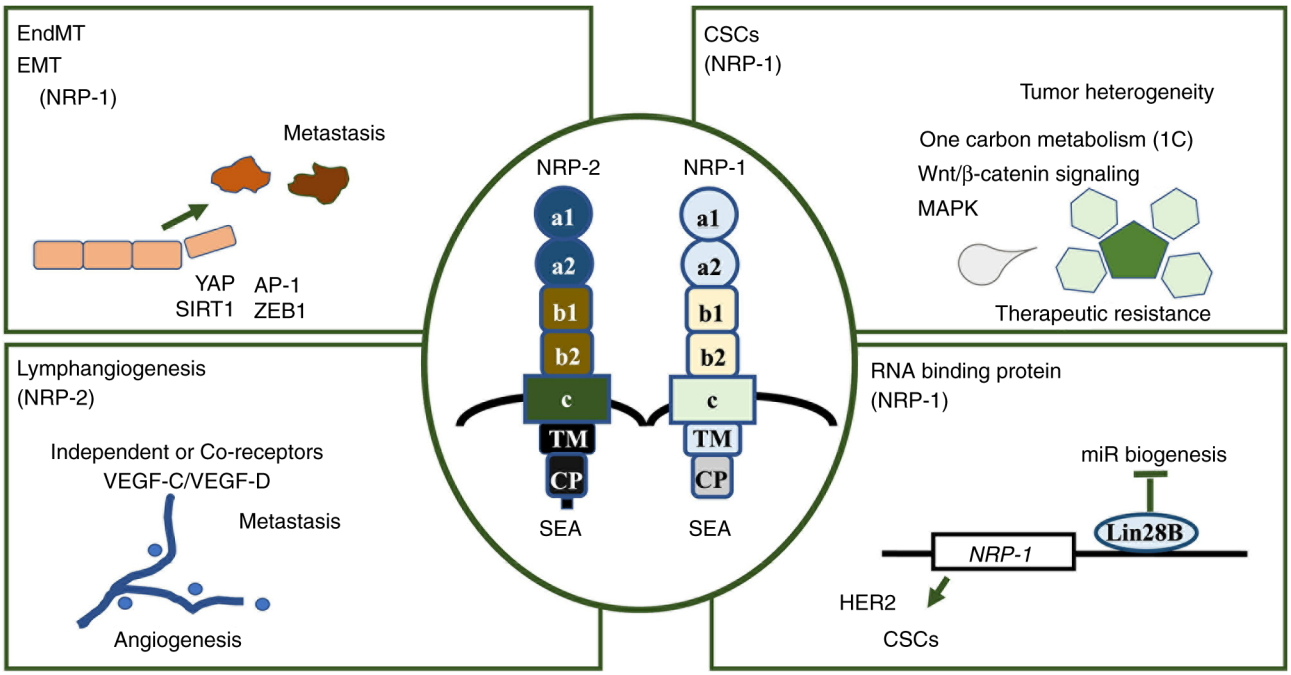

Figure 2. Biological roles of NRPs exerting various aspects. Therapeutic approaches against NRPs can be based on their involvement in various processes. NRP-1 primarily participates in the activity of EndMT and EMT, CSCs and RNA binding protein-mediated gene expression regulation, while NRP-2 is predominantly associated with lymphangiogenesis. NRP, neuropilin; EndMT, endothelial-to-mesenchymal transition; EMT, epithelial-to-mesenchymal transition; CSCs, cancer stem cells; SEA, cytoplasmic domain; VEGF-C, vascular endothelial growth factor C; VEGF-D, vascular endothelial growth factor D; YAP, yes-associated protein; AP-1, activator protein 1; SIRT1, sirtuin 1; ZEB1, zinc finger E-box-binding homeobox 1; Lin28B, Lin-28 homolog B; TM, transmembrane domain; CP, cytoplasmic region.

5. Innovative therapeutic approaches against NRPs-positive PDAC cells

EndMT. Previous studies revealed the epithelial-mesenchymal transition (EMT) mechanism by which epithelial cells lose their polarity and cell-cell adhesion and epithelial cells acquire mesenchymal features and obtain invasive phenotypes. These characterize mesenchymal stem cells, chemotherapy-resistant cells, and cancer metastasis (48,49). Extensive transcriptional reprogramming occurs during the EMT process, and this mechanism is useful for determining the presence of metastases and circulating tumor cells, as well as developing therapies against metastasizing cancer cells (50-52). In particular, high expression of zinc finger E-box binding homeobox 1, Yes-associated transcriptional regulator, FOS like 1, AP-1 transcription factor subunit (FOSL1), and the Jun proto-oncogene, AP-1 transcription factor subunit, indicate the presence of an aggressive, breast cancer subtype. These findings confirm the translational importance of the EMT process (50).

However, the EndMT process was reported to involve extensive transcriptional reprogramming in endothelial cells, shifting them toward mesenchymal phenotypes and functional responses. These processes were previously studied in cardiovascular tissues. Sirtuin 1 (SIRT1), activated by resveratrol, attenuated isoproterenol-induced cardiac fibrosis by regulating EndMT via the TGF-β1/Mothers against Decapentaplegic Homolog 2/3 (Smad2/3) pathway. Thus, TGF-β1 strongly induces EndMT. Further, SIRT1 may be involved in cardiac fibrosis under the EndMT (53-55). Tumor necrosis factor-α in cancer enhances TGF-β-induced EndMT via TGF-β signal augmentation (55).

PDAC characterizes an intense fibrotic reaction (i.e., desmoplasia) which is partly responsible for its aggressiveness, thus NRP-1 could be used to regulate TGF-β1-induced EndMT and fibrosis. Some researchers have promoted NRP-1 as a therapeutic target to reduce tumor fibrosis and slow disease progression in patients with PDAC (46). NRP interacts with many receptors and aggregates signals from other individual receptors, thereby executing EMT and EndMT (Fig. 2). Precision medicines that target NRP-1 and NRP-2 could be specified to a patient's genetic profile. Precision PDAC medicine may use drugs that target genetic mutations, such as KRAS Proto-Oncogene, GTPase, and Tumor Protein P53, and drugs that target the pathways and processes that are altered in PDAC (e.g., cell death, survival, migration, adhesion) (40,56).

Cancer stem cells (CSCs). CSCs help in therapeutic resistance and tumor heterogeneity (57,58) (Fig. 2). A study investigated the multipotent characteristics of CSCs in patients with PDAC (59). NRP signaling contributes to CSC maintenance and development (18). The VEGF/NRP signaling axis is a prime therapeutic target because of its ability to confer resistance to standard chemotherapies (18). NRP-1 interacts with PDZ (also known as disks-large homologous regions) domain-containing protein GIPC1 and PH domain-containing family G member 5 to activate p38 mitogen-activated protein kinase signaling and CSC survival (60). Targeting either NRP-1 or NRP-2 can inhibit tumor initiation and decrease therapeutic resistance in patients with cancer (18).

The increasing evidence for the NRP-1 involvement in cancer has led many studies to investigate its potential as a therapeutic target. Previous studies have focused on the

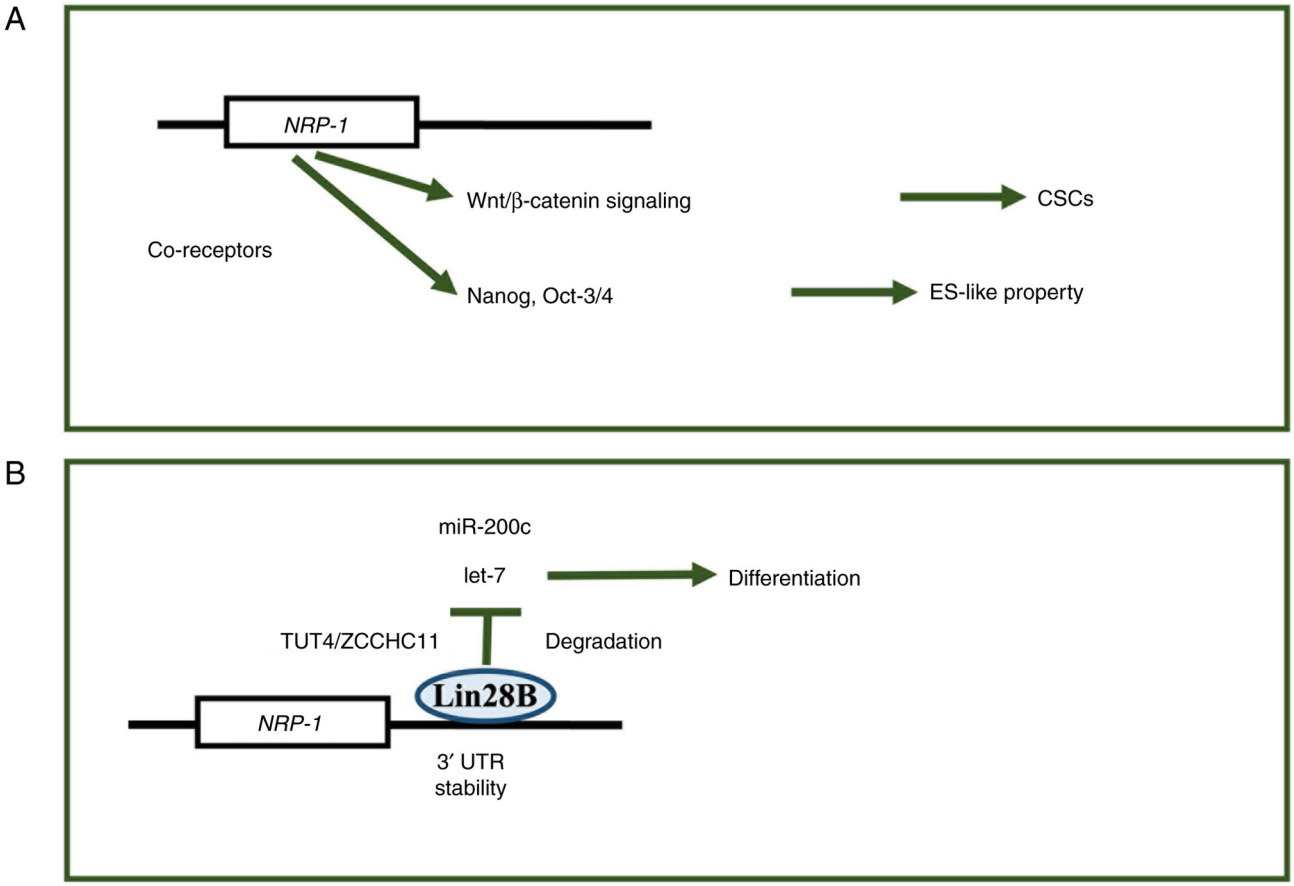

Figure 3. NRP-1 gene expression generating axis of stem-cell properties. (A) NRP-1 was involved in the Wnt/β-catenin-signaling-dependent CSC generation and the Nanog and Oct-3/4 signaling pathway mechanisms, endowing cells with biological properties resembling those of ESCs (ES-like property), such as self-renewal, pluripotency and the reversibility of cellular states, which are hallmarks of ESCs. (B) RNA binding protein, Lin28B, is involved in the control of let-7 and miRNA stability, which plays a role in controlling cell differentiation and cell stemness. NRP, neuropilin; CSCs, cancer stem cells; ESC, embryonic stem cells; miR/miRNA, microRNA; UTR, untranslated region; let-7, let-7 microRNA; TUT4, Terminal uridylyltransferase 4; ZCCHC11, zinc-finger, CCHC domain-containing protein 11; Lin28B, Lin-28 homolog B.

anticancer effects of targeting NRP-1, but little is known about the potentially adverse effects associated with such targeting. Further studies are needed to understand the full spectrum of effects associated with targeting NRP-1 in patients with cancer, including an investigation of potentially adverse events. Such studies should include both *in vitro* and *in vivo* cases and clinical trials. NRP-1 targeting-related adverse effects are important because they influence the safety and efficacy of potential future therapeutic targets.

Co-receptor targeting. Cancer cells in the tumor microenvironment produce multiple growth factors that promote lymphangiogenesis from initially enlarged lymphatics to collection within lymphatic vessels (61). Lymphatic enlargement may involve the remodeling of lymphatic vessels with smooth muscle cells (61). Several lymphangiogenic factors, such as VEGF-C/VEGF-D, can promote tumor metastasis (Fig. 2) (61).

NRP-2 acts as an independent or co-receptor for tumor lymphangiogenesis and lymphatic metastasis (Fig. 2) (62). During tumor progression, NRP-2 binds to the ligands VEGF-C/VEGF-D and activates the VEGF-C/VEGF-D/NRP-2 signaling axis, which stimulates lymphangiogenesis regulation in lymphatic endothelial cells and tumor cells (62). A 131I-labeled monoclonal antibody targeting NRP-2 for single photon emission computed tomography imaging allows lymphangiogenesis and tumor angiogenesis visualization in clinical settings (63). Reportedly, mice lacking the transmembrane receptor NRP1, also known as NRP KO mice, exhibit reduced glioma volume and decreased neoangiogenesis, while showing an increased anti-tumorigenic macrophage infiltration (64). Recent studies revealed that NRP-2 may regulate tumor progression through multiple, concurrent mechanisms (i.e., angiogenesis, lymphangiogenesis, EMT, and metastasis). These results indicate that NRP could serve as a therapeutic target for innovative antitumor therapies (62,65). First, NRPs tend to promote cell adhesion, cell-matrix interactions, cell motility, tumor angiogenesis, cell proliferation, and invasion (62,65). Second, NRPs are expressed in a range of cancer cells, including PDAC, as discussed above. Third, NRPs are amenable to targeted inhibition by inhibiting co-receptors or downstream signaling pathways (18). NRPs-ligand interaction inhibitors render NRPs an attractive target for novel therapeutic strategies (66). Preclinical studies revealed NRPs-targeting strategies to be safe, thereby further strengthening the case for their use as innovative antitumor therapies (62,65).

NRP mRNA binding protein. Recent studies open a new era of diagnostic and therapeutic that target RNA binding mechanisms of NRP transcripts. RNA binding protein Lin28B can directly bind to the 3' untranslated region (UTR) of the NRP-1 transcript, thereby increasing NRP-1 mRNA stability and NRP-1 expression (67,68). This interaction has been suggested to activate Wnt/β-catenin signaling downstream that is involved in CSC or CSC-like cell maintenance and progression in gastric cancer (Fig. 2) (68). It's worth noting that the regulation of Wnt/β-catenin signaling remains a subject of ongoing debate and investigation. While existing literature suggests an association between Lin28B-binding NRPs and Wnt/β-catenin signaling, further research is needed to fully elucidate the complexities of this relationship. Lin28B can exert multiple functions in cancer development by suppressing the biogenesis of several microRNAs, including let-7 and (possibly) miR107, miR-143, and miR-200c (69,70). Overexpressed Lin28B can recruits terminal uridylyl transferase 4 (TUT4/ZCCHC11) to pre-let-7 transcripts, leading to their terminal uridylation and degradation (71). Lin28B in cancer is indicated to be related to let-7 family derepression, which can facilitate cellular transformation with stemness. These insights contribute to the development of new strategies for cancer therapy (Fig. 3).

Another study of RNA immunoprecipitation and luciferase reporter analysis indicated that RNA binding protein PUM2 competitively bound to NRP-1 3'UTR with a microRNA, miR-376a, which can suppress breast cancer cell stemness and increase NRP-1 mRNA stability and expression in breast cancer (72).

Understanding the role of RNA binding proteins (RBPs) in cancer stemness is improving. The NRP axis is crucial for regulating key pathways that are involved in cancer progression. First, NRP-1 helps regulate the Wnt/β-catenin signaling (67,68), which is important for maintaining cancer stem-cell populations. Second, NRPs help regulate tumorigenesis and metastasis by modulating oncogenic and metastasis-associated gene expression. This is particularly true for NRP-2 and tumor lymphangiogenesis and lymphatic metastasis mechanisms (62). Third, NRP-1 promotes stem-cell-associated induced pluripotent stem gene expression, including homeobox transcription factor Nanog and POU Class 5 Homeobox 1 (Oct-3/4) (73). RBPs help regulate pre- and post-transcriptional processes, such as splicing, mRNA stability, and translation (74), thus they may contribute to cancer aggressiveness via gene expression regulation in the NRP axis.

6. Conclusions

Precision medicines that target the NRP axis might improve the diagnoses and treatment of patients with PDAC. The NRP axis contains potential therapeutic targets that could be used to develop new and individualized PDAC treatments.

Various approaches have been used to target the NRP-1 and NRP-2 axes, including gene editing, small molecule inhibitors, and monoclonal antibodies. These approaches help identify novel therapeutic targets that may improve patient outcomes and biomarkers for risk-based patient stratification, as well as the selection of the most effective treatment for each patient. Precision medicines that target the NRP axis are leading the field in an exciting new direction that may revolutionize our ability to treat this deadly disease.

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Availability of data and materials

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Authors' contributions

HI conceived the study objectives and obtained the funding. SM, TH, YT, YS, YH, YA, NG, KO, and HI contributed to creating the figures, collecting information and writing the manuscript. SM, TH, HS, ST, NG, KO, and HI constructed the study design and outlined the content. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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