

Widespread activation and critical role of EMT and stemness in the neuroendocrine differentiation of prostate cancer (Review)

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Abstract. Neuroendocrine (NE) prostate cancer (NEPC) is an aggressive and lethal subtype of prostate cancer. It is typically characterized by the expression of NE markers and the loss of androgen receptor expression. *De novo* NEPC is rare, accounting for <2% of all prostate cancer cases at diagnosis. More commonly, NEPC arises from prostate adenocarcinoma following androgen deprivation therapy, with 20-25% of metastatic castration-resistant prostate cancers undergoing NE differentiation due to lineage plasticity. During this transition, pathways associated with epithelial-mesenchymal transition (EMT) and stemness are broadly activated, which is considered to be a key driver of NEPC's high metastatic potential, resistance to chemotherapy and radiotherapy and poor prognosis. EMT facilitates metastasis by enhancing cellular motility and invasiveness, while stemness properties contribute to post-metastatic colonization, immune evasion, therapy resistance and cellular dormancy. As manifestations of cellular plasticity, these processes share overlapping molecular mechanisms. Targeting key regulators within these pathways may offer promising therapeutic strategies for NEPC.

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1. Introduction

Prostate cancer (PCa) remains the most common non-skin cancer among American men, accounting for 11% of cancer-related mortalities and ranking as the second leading cause of cancer mortality in men (1). Androgen deprivation therapy (ADT) is the primary treatment for PCa, but although patients may initially respond, the disease often recurs in a lethal metastatic form known as castration-resistant prostate cancer (CRPC) (2). Adenocarcinoma is the predominant histological variant of CRPC and continues to rely on AR signaling, which can be reactivated through acquired AR gene mutations, amplifications, or other mechanisms (3,4). In a significant subset of patients undergoing ADT, 20-25% of CRPC tumors eventually lose their dependence on AR signaling over the course of the disease, transitioning from AR-expressing PCa tissue to AR-negative, poorly differentiated small cell neuroendocrine (NE) carcinoma tissue (5). This form of PCa is commonly referred to as NEPCa, encompassing pure small cell carcinoma as well as mixed adenocarcinoma-NE tumor morphologies (6). However, newly developed NEPC is rare, observed in <2% of diagnosed PCa cases (7).

The process of NE differentiation (NED) involves extensive molecular reprogramming and is accompanied by a range of biological events. Among these, epithelial-mesenchymal transition (EMT) and stemness have attracted particular attention due to their significant roles. Stemness refers to the ability to differentiate into multiple lineages. It is evident that all these processes involve transitions in histological types, thereby introducing the concept of cellular plasticity. Cellular plasticity refers to the dynamic process by which cells transition from one state to another. The concept of 'plasticity' underscores the extensive reprogramming in stem cells that drives differentiation, highlighting the pivotal role of stemness in transforming cellular identity (8). The present study aimed not only to explore NED in prostate cancer but also to elucidate the origins, molecular mechanisms and clinical significance of EMT and stemness. It

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sought to view these three aspects as an interconnected whole within the context of prostate cancer. Although ADT therapy is the primary driver of NED transformation, the roles of EMT and stemness in this process are also significant and cannot be overlooked (Fig. 1). Prostate cancer serves as a critical platform that integrates EMT, stemness and NED. By investigating the important roles of EMT and stemness in prostate cancer, we can deepen our understanding of NED. The present study explored the extensive interconnections among EMT, stemness and NED to clarify the mechanisms of NEPC development, its clinical characteristics, molecular pathways and potential therapeutic strategies.

2. NEPC

Origin of NEPC. The origin of NEPC remains a subject of controversy. Some studies suggest that normal NE cells in the prostate may be selectively preserved and undergo malignant proliferation during ADT (9,10). However, the lineage plasticity hypothesis, which is supported by more substantial evidence, posits that prostate adenocarcinoma cells originating from luminal or basal cells may lose their adenocarcinoma characteristics and acquire a NE phenotype during prolonged ADT (9). This hypothesis suggests a similarity between the origins of NE cells and stem cells. Derivatives of therapy-resistant NE tumors consistently exhibit molecular characteristics of the primary adenocarcinoma, including specific somatic mutations (9). This supports the hypothesis that aggressive NE tumors evolve through lineage plasticity rather than originating as a second primary cancer. An open question remains as to whether lineage conversion involves a dedifferentiation phase, in which adenocarcinoma cells initially lose AR-specific gene expression and acquire stem-like properties before differentiating into NE cells, or whether transdifferentiation occurs directly, bypassing this intermediate stem-like stage (11). Based on a comprehensive analysis, the present study proposed that the development of NEPC, as illustrated in Fig. 1, may involve multiple potential origins.

The incidence of NEPC appears to be increasing, potentially linked to the use of more effective AR-targeting therapies. A rapid autopsy study found that 13.3% of patients had metastases with an AR-negative NE phenotype and an additional 23.3% had AR-negative metastases without NE features (6). These rates exceed those reported in autopsies conducted prior to the approval of potent AR signaling inhibitors such as enzalutamide and abiraterone acetate (5.4% AR-negative, 6.3% NE) (6). This suggests that with the increasing use of ADT, the progression of normal prostate adenocarcinomas to NEPC is likely to become more common.

Diagnosis of NEPC. NEPC can be somewhat imprecise. While a number of prostate adenocarcinomas contain occasional benign NE cells within the epithelium, only 5-10% of tumors exhibit distinct, large, multifocal clusters of NE cells, which are classified as adenocarcinomas NED (12). These tumors typically present as small, infiltrating glands with prominent nucleoli, hyperchromasia, absence of intraluminal secretions or pale blue mucin and isolated tumor cells, often associated with eosinophilic granules (13,14). Immunohistochemical staining for at least one NE marker, such as synaptophysin, chromogranin A, neuron-specific

enolase (NSE), or CD56, is essential for confirming the diagnosis of NE differentiation (14).

Clinical characteristics of NEPC. The association between NED and poorer oncologic outcomes in prostate cancer remains controversial. While some studies suggest a link, NED in typical adenocarcinoma has shown only a weak correlation with prognosis, insufficient to be considered clinically significant (11,13). Most research indicates that NED does not affect outcomes, with each study examining NED in prostate cancer through needle biopsies and transurethral resections of the prostate (15-18). Different pathological subtypes yield varying outcomes: NEPC tumors with well-differentiated Paneth cell-like and carcinoid differentiation tend to have an improved prognosis, while poorly differentiated NE tumors, particularly small cell carcinoma, exhibit greater aggressiveness (11).

One study compared 36 cases of pure small cell carcinoma with 51 cases (58.6%) of mixed small cell carcinoma and adenocarcinoma. The findings revealed that pure small cell carcinoma had significantly shorter overall survival compared with mixed histology (8.9 months vs. 26.1 months following NEPC diagnosis; $P < 0.001$) (19). The transition from adenocarcinoma to NEPC is not abrupt but involves an intermediate state. In its final stage, prostate cancer with NE differentiation presents the following four clinical features (6,14,19): i) Highly aggressive with poor prognosis: A systematic review found that the median survival following an NEPC diagnosis is only 7 months (20). ii) Predominantly osteolytic/visceral metastasis: Tumors tend to be large with low PSA levels, with a median PSA level of less than 4 ng/ml. iii) Non-responsive to androgen deprivation therapy. iv) Short-term response to platinum-based chemotherapy. The factors driving well-prognosed adenocarcinoma and adenocarcinoma with NED to transform into the poorly prognosed NEPC during this lineage switch remain an open question.

The role of NED in prostate cancer is closely associated with the development of castration resistance, yet the high malignancy of NEPC is more critically attributed to the molecular events that occur during NED. The extensive activation of EMT and stemness during NED probably contributes to the aggressive nature of NEPC, which is characterized by high metastatic potential, resistance to radiotherapy and chemotherapy and poor prognosis. The activation of EMT and stemness may be an indispensable component of NEPC.

3. The procedure and role of EMT

EMT is a crucial process in embryonic development; however, it also plays a pivotal role in resistance to endocrine therapy and cancer progression. EMT involves the transformation of epithelial cells into a mesenchymal phenotype, during which malignant epithelial cells progressively lose adhesion molecules such as E-cadherin, EpCAM, syndecans and tight junction molecules. At the same time, gene expression factors such as SNAIL1, SNAIL2/Slug and TWIST increase, along with mesenchymal markers such as vimentin, N-cadherin and metalloproteinases (21). Mesenchymal cells, compared with epithelial cells, exhibit enhanced migratory capacity, invasiveness, apoptosis resistance and the ability to integrate components of the Extracellular Matrix (ECM) (22). Tumors often harbor hybrid cell populations in a hybrid epithelial/mesenchymal (E/M) state,

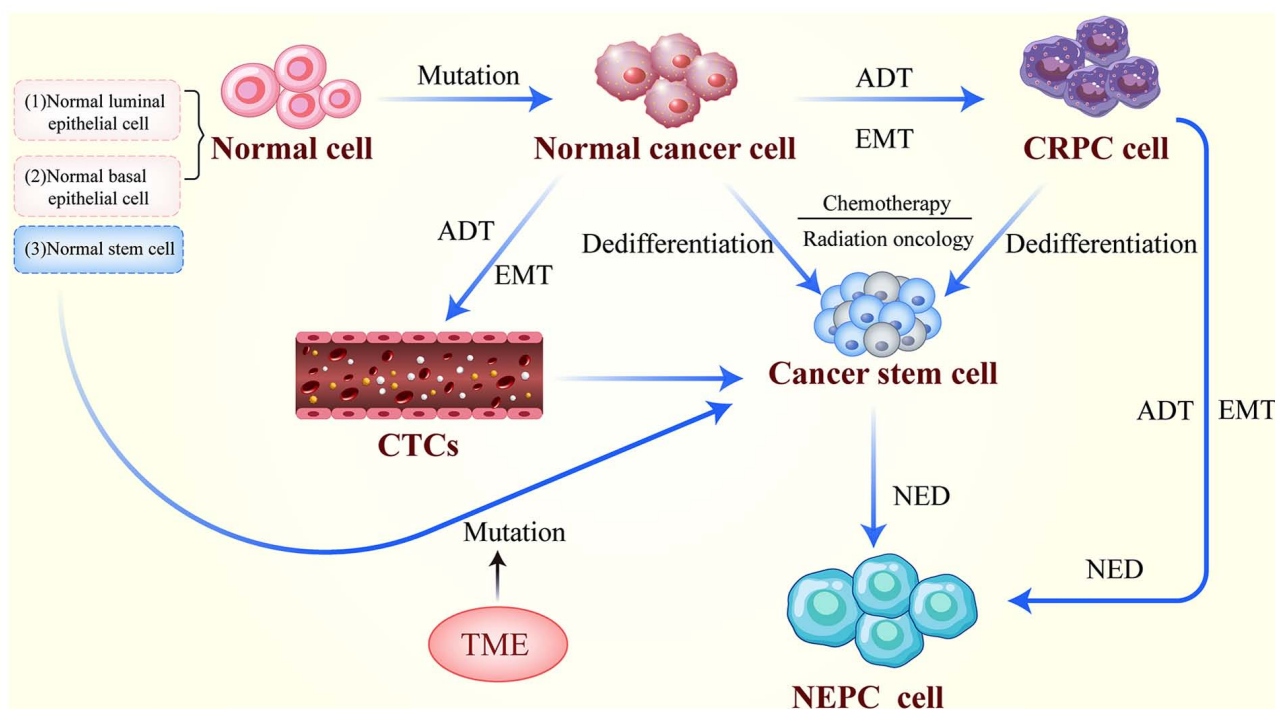


Figure 1. The development of NEPC which influenced by ADT, EMT and stemness. Normal prostate luminal epithelial cells and basal epithelial cells can undergo malignant transformation into normal tumor cells. After ADT, 10-20% of prostate adenocarcinomas transform into CRPC. With further ADT treatment, 20-25% of CRPC can transition into NEPC. During this process, while AR (androgen receptor) signaling is inhibited, the EMT process is activated and plays a critical role in castration resistance, metastasis and NED. Cancer cells often undergo dedifferentiation under the therapeutic pressure of ADT, chemotherapy and radiation oncology. CSCs may originate from malignant mutations of normal stem cells, with the TME playing a significant role in this process. Normal tumor cells can become CTCs through EMT and a small subset of CTCs survive by activating stemness, thereby acquiring immune evasion and dormancy, which gives them an opportunity to colonize. These surviving stem-like tumor cells, due to their multilineage differentiation potential, may be an important source of NEPC. NEPC, neuroendocrine prostate cancer; ADT, androgen deprivation therapy; EMT, epithelial-mesenchymal transition; CRPC, castration-resistant prostate cancer; NED, neuroendocrine differentiation; TME, tumor microenvironment; CTCs, circulating tumor cells

co-expressing both epithelial and mesenchymal traits, which enhances the metastatic potential of cancer cells, allowing them to migrate from primary to distant sites (23,24). During this reprogramming, cancer cells release enzymes that break their attachment to the basement membrane, accompanied by phenotypic alterations such as actin cytoskeleton reorganization, which promote migration and metastasis (25). While EMT facilitates the initial spread of tumor cells, the clinical emergence of metastases requires mesenchymal-to-epithelial transition (MET), which is essential for the successful seeding and colonization of disseminated cells at secondary sites (26). In NEPC, the activation of EMT may be a critical factor driving the high metastatic potential of tumors. Therefore, targeting EMT-related factors could play a pivotal role in treating NEPC. Molecules such as MCTP1 and Snail, which promote both EMT and NED, represent potential therapeutic targets (27,28).

4. Stemness

Prevalence of stem cells and tissue colonization. The most lethal malignancies, including lung, gastric, liver, breast and prostate cancers, as well as hematological cancers such as leukemia, contain cells with varying stem cell-like properties (29). As early as 1877, Cohnheim, a student of Virchow, observed this population of cells with embryonic characteristics (30). Today, these cells are referred to as cancer stem cells (CSCs) or tumor-initiating cells (TICs) and are

recognized as the driving force behind tumor establishment and progression (31). Although CSCs comprise <1% of the primary tumor (32,33), their effect is profound. Stemness is characterized by the capacity for self-renewal, multilineage differentiation and extended cellular lifespan functions vital for normal physiology and tissue repair (34). As with normal stem cells, cancer stem cells have the ability to divide, grow, undergo clonal expansion and differentiate (35). This ability closely mirrors the uncontrolled proliferation observed in cancer cells, making CSCs genetically akin to cancer cells and more susceptible to mutations that drive oncogenesis. However, unlike normal stem cell niches, which possess tumor-suppressive functions and various signaling pathways to regulate growth, CSC niches provide a supportive microenvironment for cancer cells. This niche includes cancer-associated macrophages and cancer-associated fibroblasts, which promote cancer cell growth and differentiation through the secretion of various molecules, including growth factors, cytokines and vesicles such as exosomes (36,37). CSCs can adapt to stress conditions such as oxidative damage, hypoxia and exposure to exogenous substances by altering their gene expression programs, leading to phenotypes associated with migration, invasion and drug resistance (38,39).

Current evidence suggests that the primary reservoirs of normal stem cells in the prostate are luminal and basal cells (24). Under normal conditions, stem cells typically exhibit a basal cell phenotype (40). However, following ADT,

stem-like cells with a luminal phenotype are more likely to serve as the origin of tumor re-initiation in CRPC (41). The cellular origin of NEPC is also luminal epithelial cells (9) and the stemness of these luminal epithelial cells may facilitate NED.

Acquisition of stemness and resistance to therapy. Tumors acquire stemness under the pressure of endocrine therapy, chemotherapy and radiotherapy through a series of related molecular mechanisms. During chemotherapy or radiotherapy, certain tumor cells may enter a state of senescence known as therapy-induced senescence (TIS), with the senescence-associated secretory phenotype (SASP) promoting the emergence of stem cell-like subpopulations. Over time, the antitumor effects of TIS may wane, allowing the cancer to regain stemness, which can contribute to tumor recurrence (39). Research has shown that the tumorigenic potential of cancer cells is linked to the increased expression of CD44 or other CSC-like characteristics, driven by SASP-related cytokines such as IL-8 and IL-6 (42). The acquisition of cancer stemness is driven not only by the evasion of senescence but also by the plasticity of non-CSCs and CSCs. This plasticity is influenced by endocrine therapy, where the loss of AR signaling can induce EMT and NED, processes that further promote stemness. The effects of SASP and plasticity are difficult to distinguish, with key interleukins involved in SASP being IL-1, IL-6 and IL-8, which can influence the plasticity phenotype of CSCs (43). Additionally, the development of CSCs remains a topic of ongoing debate, with various models proposed. Genetic and epigenetic alterations, along with changes in the 'stem cell niche', can result in unchecked stem cell proliferation, endowing these stem-like cells with oncogenic and drug-resistant potential (39). CSCs may arise from aberrant differentiation of stem cells, mutations and dedifferentiation of normal cells, or dedifferentiation of tumor cells.

CSCs exhibit resistance to androgen deprivation, pro-oxidants, radiation and chemotherapy (44-46). In other studies, CSCs have demonstrated significant resistance to various treatments, including tyrosine kinase inhibitors, taxanes and topoisomerase inhibitors (47,48). The resistance of CSCs to chemotherapeutic agents arises from a combination of intrinsic and extrinsic factors (49). Intrinsic factors contributing to CSC therapy resistance include efficient slow cell cycle progression, DNA repair mechanisms, tumor cell vasculogenic mimicry, active anti-apoptotic pathways, poor immunogenicity and the maintenance of stemness characteristics. Extrinsic factors involve the high cell density of CSCs, lineage plasticity, the tumor microenvironment (TME) and epigenetic influences (31,50-52). These features are also evident in NEPC. Although NEPC initially shows sensitivity to platinum-based chemotherapy, resistance and disease progression often develop rapidly and the response to other types of drugs is generally poor. Additionally, similar to stem cells, NEPC exhibits alterations in DNA repair mechanisms (53), tumor angiogenesis (54,55), anti-apoptotic pathways (56) and autophagy (57,58).

Stemness and tumor metastasis. The relationship between CSCs and tumor metastasis is complex, with evidence suggesting that the role of stemness in metastasis is not to promote the initiation of

metastasis, but rather to facilitate post-migration colonization (38). Current research indicates that EMT promotes the emergence of stemness (59), while stemness may, in turn, facilitate MET, aiding in cellular colonization; a coherent process. Mesenchymal cells lack the ability to colonize; however, CSCs, which constitute a small subset of malignant cells within most tumors, are ultimately responsible for relapse and metastasis (38). Epithelial cell adhesion molecule (EpCAM) is one of the earliest identified CSC markers. The expression of EpCAM is associated with a CSC-like phenotype that promotes bone metastasis in mice and is often associated with the expression of other CSC markers, such as CD44 and CD166 (60). Additionally, as an epithelial cell marker, EpCAM is downregulated during EMT and upregulated during MET, with larger metastatic tumors (≥ 30 cells) and primary tumors showing high levels of EpCAM expression (61). Therefore, the expression of EpCAM in CSCs reflects their potential for adhesion and post-migration colonization, which is associated with MET. In a mouse model of basal-like breast cancer, continuous MET activation induces stem cell properties (62). In pancreatic and prostate cancers, both MET and hepatocyte growth factor are predominantly expressed in stem-like tumor cells (38). Studies have shown that hematopoietic stem cells undergo a phenotypic shift toward a more mesenchymal state when multiple stemness genes such as Sox2, Myc and Klf4 are knocked out (38,46). By using magnetic-activated cell sorting, CSC populations have been isolated from mesenchymal cancer cells (MCCs). Compared with MCCs, CSCs demonstrate slower proliferation, greater resistance to apoptosis, drug resistance and heightened clonogenic potential, although their invasiveness is reduced (38).

CSCs and disseminated tumor cells (DTCs) share several biological characteristics, such as dormancy (63). Research indicates that DTCs expressing a CSC phenotype constitute only a small fraction of the total DTC population (63). However, these CSC-like DTCs are more effective at displacing hematopoietic stem cells (HSCs) from their interaction with osteoblasts a mechanism HSCs use to localize to the bone niche (64). By displacing HSCs, CSCs bind to components of the HSC niche, enriching the dormant population of prostate cancer cells and enhancing their ability to resist various chemotherapeutic attacks (65,66). Dormant-enriched human PCa cell populations bind to osteoblasts within the bone marrow HSC niche, inducing the expression of TANK-binding kinase 1 (67). This, in turn, inhibits mTOR signaling, promoting dormancy, maintaining the CSC phenotype (CD44+/CD133+) and contributing to drug resistance (61). Increasing evidence suggests that CSCs, though a small population within most tumors, are largely responsible for relapse and metastasis (38,68). Therefore, the activation of stemness in NEPC may promote the survival and colonization of tumor cells after metastasis, leading to the development of visceral and osteolytic metastases (14).

5. Interactions between ADT, EMT, stemness and NED

ADT promotes EMT, stemness and NED in prostate cancer. It is important to determine whether these three processes occur simultaneously or follow a specific sequential order. After reviewing a large body of literature, the present study proposed that the relationship among these processes can be simplified as shown in Fig. 2. The findings suggested that EMT occurs earlier than stemness, which, in turn, precedes

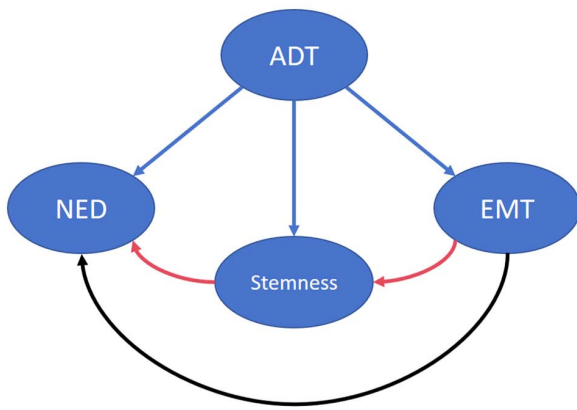


Figure 2. The simplified association among ADT, EMT, stemness and NED in prostate cancer. ADT concurrently promotes EMT, stemness and NED; EMT facilitates stemness and NED; and stemness drives NED. ADT, androgen deprivation therapy; EMT, epithelial-mesenchymal transition; NED neuroendocrine differentiation.

NED. The evolution from androgen receptor-positive prostate cancer (ARPC; AR+, NE-) to NEPC (NE+, AR-) often involves intermediate states, including AR-, NE- and AR+, NE+ subtypes. Studies indicate that mesenchymal and stem-like prostate cancer (MSPC) overlaps with the AR-, NE-subtype by 75-80%, suggesting that MSPC is an intermediate state in the progression from ARPC to NEPC (69). EMT may occur prior to the acquisition of stemness, as the EMT process can induce a stem-like state due to the partial overlap between EMT and stemness transcriptional programs (59). The role of EMT in conferring stemness mirrors its function in normal embryonic development, where EMT primarily controls the differentiation of stem cells into various lineages (70). Furthermore, molecules associated with EMT and stemness collectively promote the progression to NED.

ADT promotes EMT, stemness and NED. Induced by ADT and inversely correlated with AR signaling, features such as EMT, CSCs and NED are commonly observed (24,71,72). These features emerge under the stress of ADT treatment, with tumor cells undergoing transdifferentiation to evade drug-related pressures (73). For example, LuCaP 35 xenografts derived from human PCa show concurrent EMT and increased expression of stem cell markers such as WNT 5a and WNT 5b upon ADT treatment (73). Androgen deprivation can also induce the upregulation of EMT-associated molecules, including vimentin (VIM), Zinc finger E-box binding homeobox 1 (ZEB1), N-cadherin (CDH2), Snail Family Zinc Finger 2 (SLUG) and Twist Family BHLH Transcription Factor 1 (TWIST1) (73). Notably, a bidirectional negative feedback loop exists between AR and ZEB1. After ADT treatment, stem-like cells reach their peak levels (74). PSA-/low cell populations exhibit gene expression profiles similar to those of stem cells, characterized by enhanced self-renewal capacity and resistance to ADT and chemotherapeutic agents (75). CSCs isolated from AR-negative DU145 cells show high expression of stem cell markers, including CD24, integrin $\alpha 2 \beta 1$, CD44 and the reprogramming factor SRY-box 2 (SOX2). These cells display tumor-initiating potential and self-renewal capabilities (76). These changes may represent adaptive mechanisms under the

pressure of ADT treatment, driving the tumor cells toward similar pathways.

ADT suppresses AR signaling, leading to a transformation of epithelial cells into a NE phenotype, which is widely accepted as a key process in prostate cancer progression. The focus here is on the downstream molecular mechanisms through which ADT-induced AR suppression promotes NED. ADT enhances NED by increasing the expression of Forkhead transcription factor (Fox)a2 and knockout of Foxa2 reverses the conversion of the glandular to the NE lineage (77). Notably, ten days after enzalutamide treatment, significant increases in chromatin accessibility were observed compared with 3 days and untreated CRPC, with enrichment in stem cell and neuronal pathways. Knockout experiments further confirmed that ASCL1 is essential for establishing neuronal and stem cell-like lineages (78). ASCL1 and FOXA2 are both considered lineage-determining factors in NED, as they remodel chromatin into a permissive state, facilitating subsequent transcription factor binding and indirectly highlighting the critical role of ADT in NED (79). ADT alleviates AR-mediated suppression of H19, a long non-coding (lnc)RNA involved in cellular plasticity, driving either the NE phenotype (H19 overexpression) or the luminal phenotype (H19 knockdown) (80). Furthermore, ADT reduces EHF transcription by disrupting AR binding to androgen response elements, thus promoting the expression and enzymatic activity of Enhancer of Zeste Homolog 2 (EZH2), which catalyzes histone H3 trimethylation at lysine 27 to repress downstream genes and facilitate NED (81). Enzalutamide promotes lncRNA-p21 transcription through AR inhibition, altering EZH2 function from a histone methyltransferase to a non-histone methyltransferase, leading to STAT3 methylation and promoting NED (82). ADT also activates cAMP response element-binding protein (CREB) and the CREB-EZH2-TSP1 pathway is a newly identified mechanism driving therapy-induced NED in prostate cancer (55). Overexpression of DPYSL5 in prostate cancer cells induces a neuron-like phenotype, enhances invasion and proliferation, upregulates stemness and NE-related markers and negatively correlates with AR and PSA (83). Mechanistically, DPYSL5 promotes prostate cancer cell plasticity via EZH2-mediated polycomb repressive complex 2 (PRC2) activation (83). Depletion of DPYSL5 reduces proliferation, induces G₁ phase cell cycle arrest, reverses the NE phenotype and upregulates luminal genes. Notably, EZH2 emerges as a key downstream molecule in these ADT-induced NED pathways. Enzalutamide also induces NED via the MAOA/mTOR/HIF-1 α signaling axis (84). Additionally, AR inhibition depletes REST, leading to NED (85). BRN2 expression, directly suppressed by AR and negatively correlated with AR activity, drives the NE phenotype by binding to SOX2 upon release from suppression (86). AR binds directly to the cis-enhancer region of SOX2 and AR inhibition enhances SOX2 expression (87). Following AR pathway inhibition (ARPI), the expression of PEG10 is derepressed, regulated by both AR and the E2F-RB1 transcription factor pathway during the early and late stages of NEPC development (88). Targeting these downstream pathways associated with AR inhibition may provide opportunities to reverse EMT, stemness and NED in prostate cancer.

EMT promotes stemness. Various EMT-related molecules, including ZEB1, PDGF-D, Snail and E-cadherin, play

pivotal roles in promoting stemness in cancer cells (24). The relationship between stemness and EMT is complex. For example, silencing E-cadherin in spheroids derived from the PC3 human prostate cancer cell line stimulates the EMT process (89), whereas its expression induces stemness gene expression and spheroid formation in DU145 prostate cancer cells (90,91). Elevated levels of the EMT regulator ZEB1 promote stem cell-like traits in prostate cancer cells, leading to increased SOX2 expression (92). Additionally, CD44-low cells (non-CSCs) can transition to a CD44-high phenotype (CSCs), forming mammospheres in response to TGF- β -driven ZEB1 expression (93). Overexpression of platelet-derived growth factor D in PC3 cells results in morphological changes characteristic of EMT, enhanced clonogenicity and increased spheroid formation, along with higher expression of stemness-related transcription factors, including Sox2, Oct4, Nanog, Lin28B and components of the PRC2 (94). Loss of E-cadherin is crucial in conferring oncogenic properties, particularly in enhancing both stemness and metastatic potential (95). Activation of TGF- β signaling or transcription factors such as Snail can convert non-cancer stem cells (CD133- or ALDH-low) into cancer stem cells/progenitor cells (CD133+ or ALDH-high) (96).

EMT promotes NED. Recent evidence underscores the crucial role of EMT as a driving force in NED. Key EMT inducers involved in NED, such as Twist, Snail, Slug and E-cadherin repressors such as Zeb1/2, are essential to this process (97). For instance, overexpression of Snail leads to the downregulation of E-cadherin and the upregulation of NE differentiation markers, including ENO2 and CHGA (98). The interplay between the TGF- β signaling network and the AR axis markedly influences EMT-related phenotypic changes. Disruption of epithelial homeostasis via EMT facilitates the transformation of epithelial-derived tumors into invasive forms, enabling them to acquire a NE phenotype and rapidly metastasize (99).

Stemness-related factors promote NED. Through extensive literature review, the present study identified that stemness-related factors play a crucial role in the process of NED. Particularly, molecules such as SOX2, MYC, EZH2 and ASCL1.

SOX2, a member of the SRY-related HMG box family, is essential for maintaining embryonic stem cell pluripotency and regulating neuronal differentiation (100-102). Compared with patients with PCa or CRPC-adenocarcinoma, NEPC patients exhibit significant upregulation of SOX2 expression (103). In PCa cells, NED genes are upregulated by SOX2 overexpression and downregulated by its silencing (103). These findings suggest that the loss of PTEN, TP53 and RB1 drives the NE phenotype through SOX2 (104). The molecular mechanism by which SOX2 promotes NED likely involves transactivation of Serine Peptidase Inhibitor Kazal Type 1 (SPINK1) and upregulation of H19 and ASCL1 (105). Additionally, SOX2 regulates global epigenetics through LSD1, leading to reduced ARPC-specific gene expression in NEPC, representing a novel mechanism for SOX2 in NEPC progression (106). Several signals upregulate SOX2 in NEPC. AR chromatin immunoprecipitation (ChIP) studies in CRPC-adenocarcinoma cells (CWR-R1) show that AR directly binds to the enhancer region of SOX2 and

ligand activation of AR inhibits SOX2 expression, which is relieved by ADT, eliminating AR ligand-mediated suppression of SOX2 expression (107). RB1 can interact with E2F in fibroblasts and be recruited to the promoter region of SOX2, marking it for repression (108). The core pluripotent stem cell gene LIN28 mediates the derepression of the transcription factor HMGA2, which in turn promotes SOX2 expression by inhibiting let-7 miRNA expression (109). Overexpression of Mucin 1-C-terminal subunit (MUC1-C) induces SOX2 expression through the MUC1-C-MYC-BRN2 axis (110). TBX2 binds to the SOX2 promoter and inhibits the expression of miR-200c-3p, leading to increased expression of its targets, SOX2 and N-MYC (111). Furthermore, SNAI2, TRIM59, TRIB2 and SRRM4 all promote NED by upregulating SOX2 expression (92-95). Overall, SOX2 serves as a critical downstream mechanism in multiple molecular pathways leading to NED. Additionally, the pluripotency factor Oct4 is closely associated with AR-independent NE-like tumors (112).

The MYC protein family plays a significant role in cell reprogramming. As a major transcriptional regulator, MYC promotes a plasticity-permissive, stem-like state. Inhibition of CDC7 suppresses NE transdifferentiation by inducing the degradation of MYC protein, enhancing the response to targeted therapy in *in vivo* models of NE transformation. This process is linked to both stemness and histological transformation (113). Additionally, CDC7 inhibition markedly prolongs the response to standard cytotoxic agents, such as cisplatin and irinotecan, in small cell prostate cancer models (113). The N-Myc oncogene, encoded by the MYCN gene, is a neurodevelopmental-related transcription factor. N-Myc expression is higher in NEPC compared with non-NE CRPC and it plays an important role in NED (114). In the absence of androgens, upregulation of N-Myc is associated with an enrichment of stem cell characteristics and neural lineage differentiation markers. In castrated 22Rv1 xenograft mice and GEM models, the N-Myc signature was enriched with genes related to neural lineage pathways, such as neural progenitor cell bivalent genes and genes involved in neural development (such as SOX11, SOX21, NTRK1 and NKX2-1), adult stem cells (such as HOXA2/A3/A9/A10 and WNT5A), ESCs (such as SOX2) and NEPC (such as CHGA), while epithelial lineage-associated genes were downregulated (115). Studies on mouse models with PTEN deficiency and MYCN overexpression have shown that N-Myc interacts with EZH2, AR and various AR co-factors (such as FOXA1 and HOXB13) both *in vitro* and *in vivo* (114-116). N-Myc directly binds to the promoters of NSE, SYP and AR, suggesting its critical role in regulating the emergence of NEPC (116). Furthermore, N-Myc interacts with AR by directly binding to AR enhancers, driving NE differentiation through EZH2-mediated transcriptional programs (114). Aurora A stabilizes N-Myc (117) and the Aurora A inhibitor alisertib can disrupt this stabilization, preventing NED (118). Another member of the MYC family, c-MYC, collaborates with Pim1 kinase to drive NE differentiation in aggressive prostate tumors in prostate xenografts (119).

ASCL1, an early lineage determinant of NED, is enriched in regions associated with DNA-binding motifs involved in stem cell and neuronal lineage programming (78,120). Genome-wide mapping of ASCL1 occupancy in NEPC cell lines through chromatin immunoprecipitation sequencing

revealed that ASCL1 directly regulates key CSC genes, including SOX2, NANOG and OCT4, as well as NE genes such as CHGA, ENO2 (NSE), NCAM1 and DLL1, all known ASCL1 targets (78). Further supporting this, knockdown of ASCL1 resulted in downregulation of these genes, while deletion of ASCL1 triggered a lineage switch from neuronal to luminal (78,120). Additionally, the ASCL1 cistrome is enriched with targets of PRC2 and deletion of ASCL1 reduces EZH2 activity in NEPC cell lines. Conversely, overexpression of ASCL1 enhances EZH2 activity (78).

EZH2, an epigenetic regulator and catalytic subunit of PRC2, catalyzes the trimethylation of histone H3 at lysine 27 (H3K27me3), promoting transcriptional silencing. Its catalytic activity contributes to lineage-specific cell fate determination by either repressing lineage-specific factors or activating transcription factors that drive stem cell and NE programs (121). Compared with adenocarcinoma and a number of progressing NEPC patients, EZH2 is overexpressed in NEPC samples (121,122). It interacts with miR708 to drive the NE phenotype in PCa (123). In an N-Myc-driven NEPC mouse model, EZH2 catalytic activity facilitates the formation of an N-Myc/AR/EZH2-PRC2 complex, downregulating AR signaling and promoting NEPC (114). Furthermore, AR and EZH2 co-occupy the reprogrammed AR cistrome to transcriptionally modulate stem cell and neuronal gene networks (124). EZH2 also plays a crucial role in promoting NE differentiation during ADT, as outlined in previous sections.

The stem cell marker CD44 is selectively associated with NE tumors, with CD44+ PSA-cells capable of initiating tumors, developing castration resistance and expressing NE markers (75). Recently, the FOXC2 has been identified as a unique marker of the stem cell phenotype in NEPC, where AR+ prostate cancer cells acquire FOXC2 expression, correlating with NE transdifferentiation and resistance to enzalutamide and docetaxel (125). Loss of FOXC2 function is sufficient to trigger a phenotypic switch from NE to luminal epithelial characteristics. O-linked N-acetylglucosamine transferase (OGT), associated with stemness and EMT, is crucial for KIF1A-induced NE phenotype and invasive growth (126). MYBL2, a pluripotency gene, serves as a chromatin-binding partner for key pluripotency factors such as OCT4, NANOG and SOX2. Its expression and activity are enriched in human NEPC and NE-like mouse models (127). Cyclin-dependent kinase 2, a transcriptional target of MYBL2, exhibits potent anti-tumor responses when inhibited in RB1-deficient NEPC (128). Furthermore, L1CAM knockdown in PC3 cells impairs their ability to form tumor spheres and reduces the expression of both cancer stemness and NE markers (129).

6. Shared molecular mechanisms

Numerous classical molecular pathways, genes, transcription factors and epigenetic abnormalities can simultaneously activate EMT, stemness and NED (Table I). These phenomena exhibit broad associations, demonstrating the consistency and synergy among the three processes. A number of studies have shown that blocking these key molecules can reverse NED differentiation and inhibit tumor progression *in vivo*,

suggesting that targeting these molecular pathways may offer meaningful therapeutic opportunities for advanced NEPC patients and potentially lead to improved treatment outcomes (114,130-133).

Key genes such as PTEN, TP53 and RB1, which play pivotal roles in NED, are also closely associated with stemness and EMT. Mutations or deletions in the TP53 gene are present in 66.7% of CRPC-NE and 31.4% of CRPC-Adeno patients (134). TP53 acts as a suppressor of genes involved in stemness, including Sox2, Oct4, Nanog and c-Myc (135,136). Thus, mutations and deletions in TP53 can lead to the activation of stemness. PTEN also plays a critical role in regulating normal and CSC homeostasis, with its loss promoting CSC expansion in both solid and hematologic malignancies (137). The loss of TP53 and PTEN induces an increase in the NE phenotype, with NE differentiation in TP53- and PTEN-deficient CRPC being partially mediated by SOX11 (138).

RB1 loss is present in 70% of NEPC samples compared with 32% of CRPC-Adenocarcinoma samples (139). Rb, the protein encoded by RB1, can bind to E2F proteins to form the Rb/E2F complex and interactions through the E2F-binding pocket are essential for the role of Rb in silencing pluripotency genes. The Rb/E2F complex binds to gene promoter regions, directly repressing the expression of pluripotency factors, including SOX2, Oct4, Nanog, KLF4 and TCF3. Loss of Rb leads to dissociation and inactivation of the Rb/E2F complex, resulting in the loss of inhibition on pluripotency genes such as SOX2. ChIP analysis following Rb loss reveals significant changes in histone modifications across the genome, with increased levels of H3Ac and H4K4me3 and decreases levels of trimethylation of H3K27me3 in multiple pluripotency genes, such as Oct4, Nanog and SOX2, leading to enhanced chromatin accessibility (108,140). After dissociation, E2F becomes activated and promotes the expression of target genes. For example, E2F1 induces RACGAP1 expression and RACGAP1 stabilizes EZH2, a component of the PRC2 pathway, thereby promoting NED in prostate cancer (110,140-141). In gene-modified mouse models, the individual loss or mutation of PTEN, TP53, or RB1 does not lead to NEPC (138,142). However, the simultaneous loss or mutation of any two of these tumor suppressor genes, RB1, TP53 and PTEN, markedly elevates the incidence of NEPC (130,131,138,142). Gene set enrichment analysis shows that the expression of E2F target genes and NE lineage genes is altered in tumors with dual-gene and triple-gene deletions, with increased expression of stemness-related genes such as SOX2 and EZH2. This may be closely related to the dissociation of the Rb/E2F protein complex or due to the loss of TP53 inhibitory effects. By inhibiting EZH2, AR expression in dual-gene mutant tumor cells can be increased and SYP expression can be decreased (130). Knocking out SOX2 can also reverse lineage conversion and restore resistance to enzalutamide (131). This highlights the important role of stemness-related programs in promoting NE differentiation in prostate cancer.

MUC1-C is a representative molecule that concurrently regulates EMT, stemness and NED. MUC1 is a transmembrane mucin glycoprotein expressed on the apical surface of normal epithelia, consisting of two

Table I. Shared pathway and molecular of EMT, stemness and NED.

A, Signaling pathways			
Name	Role	Mechanisms	(Refs.)
JAK/STAT	EMT	1. JAK-STAT3 phosphodimerization-EMT (Twist, MMP2, 9 and 7)	(154-159)
		2. IL-6-JAK/STAT3-EMT	
		3. CCL3-JAK/STAT3-EMT	
		4. TGF- β 1-JAK/STAT3-Twist-E-Cadherin $\downarrow$	
		5. TP53-/RB1-JAK/STAT3-EMT	
		6. snail-JAK/STAT3-EMT	
	stemness	1. JAK/STAT3-Oct4 and SOX family transcription factor	(154,158,160)
		2. TP53-/RB1-JAK/STAT3-stemness	
		3. AJAP1 $\downarrow$ -JAK/STAT3-stemness	
	NED	1.IL-6-JAK/STAT3-NE maker(CHGA and ENO 2)	(154,158,161,162)
		2.IL-8-JAK/STAT3-NED	
		3. TP53-/RB1-JAK/STAT3-NED	
		4. IL-6-PEDF-RhoA-NF- κ B-STAT3-NED	
		5.mTOR or MAPK pathway-STAT3 phosphodimerization-AR $\downarrow$	
		6. IL-6-JAK/STAT3-AR $\downarrow$	
PI3K/AKT/mTOR	EMT	PI3K/AKT/mTOR-EMT	(163-165)
	stemness	pAKT-H3K9 acetylation $\downarrow$ -CSCs	(163,164,166)
	NED	PI3K/AKT/mTOR-AMPK-NED	(167)
Hedgehog pathway	EMT	Hedgehog-TGF- β 1	(168,169)
	stemness	Hedgehog-the transforms and maintains of CSCs	(170-172)
	NED	Hedgehog $\downarrow$ -PTCH-SMO $\downarrow$ -AR $\downarrow$ -NED	(173)
Wnt pathway	EMT	1.Wnt/ β -Catenin-EMT	(174,175)
		2.PRKAR2B-Wnt-EMT	
		1.Wnt signaling enhances CSC renewal and expansion	
		2.DAB 21 P $\downarrow$ -Wnt/ β -Catenin-CSC	
	NED	3.ESM1- β -catenin-tcf4-stemness	(176-179)
		4. KIT-Wnt/ β -catenin-Stemness	
		1.Wnt or AR $\downarrow$ -Wntless (WLS)-ROR2/PKC δ /ERK-NED	
		2.AR $\downarrow$ -Wnt-11-NSE and ASCL1-NED	
		3.AR $\downarrow$ -YY1-FZD8-Wnt9A-FYN/STAT3-NED	
		4.Wnt/ β -Catenin-TCF4-NED	
Notch	EMT	5. ADT-TCF7L1-Wnt4-IL-8/CXCR2-NED	(180-186)
		6. AR $\downarrow$ -PCDH-PC-Wnt-NED	
	stemness	7.Wnt-EZH2-microRNA-708 $\downarrow$ -NED	(187,188)
		8. ALK/N-Myc-Wnt/ β -catenin-NED	
	NED	1.Notch-EMT	(189)
		2.Notch-HIF-1 α -SNAI1	
	TGF- β	Notch-stemness	(190-192)
		DLL3-Notch 1 and Notch 2 $\downarrow$ -Hes1 and Hey1 $\downarrow$ -NED	
	EMT	TGF- β -PI3 K/AKT-EMT	(193)
	stemness	TGF- β -Smad-c-MYC and SPOP $\downarrow$	(194,195)
p38 MAPK	NED	IL-6-TGF- β -SMAD2-p38MAPK-NED	(196)
	EMT	CAF-IL32- β 3-p38 MAPK-EMT	(197)
	stemness	TPTEP1-MAPK14-P38 MAPK-stemness $\downarrow$	(198)
	NED	p38 MAPK-FOXC2-NED	(125)

Table I. Continued.

B, Genes			
Name	Role	Mechanisms	(Refs.)
MAOA	EMT	CAFs-MAOA/mTOR/HIF-1 α -EMT	(199)
	stemness	MAOA-stemness	(84,200)
	NED	AR $\downarrow$ -MAOA-mTOR-HIF-1 α -NED	(84)
TP53	EMT	1.TP53 $\downarrow$ -EMT(E-cadherin, β -catenin, vimentin, fibronectin, slug, snail)	
		2. Wild-type p53-miR-145-EMT $\downarrow$	(201-205)
	stemness	1. Wild-type p53-miR-145-stemness $\downarrow$	
		2.TP53-Nanog, Sox2, Oct4 and c-Myc $\downarrow$	(135,136,204,205)
	NED	1.LSD1-TP53 $\downarrow$ -NED	
		2.TP53-/RB1-SOX2 or EZH2-NED	(131,206)
RB1	EMT	RB1 $\downarrow$ -EMT	(207)
	stemness	RB1 $\downarrow$ -E2F-Pluripotency factors (Oct4, Nanog, Sox2, KLF4, TCF3) and EZH2	(110)
	NED	TP53-/RB1-SOX2 or EZH2-NED	(131)
PTEN	EMT	PTEN $\downarrow$ -PI3K/Akt-EMT	(207,208)
	stemness	PTEN $\downarrow$ -PI3K/Akt-stemness	(207,208)
	NED	TP53-/PTEN- or PTEN-/RB1-NED	(209)
C, Transcription factors			
Name	Role	Mechanisms	(Refs.)
FOXA2	EMT	FOXA2-EMT	(27)
	stemness	EGFR-ERK-FOXA2-SOX9-stemness	(210)
	NED	1.ZBTB46-FOXA2 and HIF1A-MCTP1-SNAI1-NED	
		2.PHF8-FOXA2-NED	
		3.Siah2-HIF-1 α -FOXA2-Hes6, Sox9 and Jmjd1a-NED	
		4.FOXA2-KIT-NED	(27,211-214)
SOX2	EMT	SOX2-SPINK1-EMT	(105)
	stemness	1.SOX2 is the cell maker of stem cell	
		2.SOX2-SPINK1-stemness	(105)
	NED	1.SOX2-SPINK1-NED	
		2.SOX2-LSD1-NED	
		3.SOX2-ASCL1-NED	
		4.ADT-SOX2-H19-NED	
		5.SRRM4-SOX2-NED	
		6.LIN28B-et-7-SOX2-HMGA2/HMGA2-NED	
		7.TRIM59-RB1-/P53-SOX2-NED	
		8.TRIB2-BRN2-SOX2-NED	
		9.TBX2-miR-200c-3p-SOX2-N-MYC-NED	(80,87,105,106, 109,111,215-218)
PEG10	EMT	PEG10-TGF- β -Snail	(88)
	stemness	-	
	NED	1.RB1-/TP53-PEG10-NE maker	
		2.ONECUT2-PEG10-NED	(219-221)
ASCL1	EMT	ASCL1-Wnt11-E-cadherin	(222)
	stemness	ASCL1-SOX2, NANOG and OCT4-stemness	(78)
	NED	1.ASCL1-CHGA, ENO2 (NSE), NCAM1, DLL1-NED	
		2.ASCL1-CEACAM5-NED	
		3.NOTCH-ASCL1-RB-p53	(78,223,224)
MYC	EMT	TM4SF1-Wnt/ β -catenin-c-MYC/SOX2-stemness and EMT	(225)
	stemness	c-MYC and N-MYC are the cell maker of stem cell	(45)
	NED	1.c-MYC/Pim1-NED	
		2.N-MYC-EZH2-AR $\downarrow$ -NED	
		3.N-MYC-SYP and NSE	(114,115,119)

Table I. Continued.

D, Epigenetics				
Name	Role	Mechanisms	(Refs.)	
EZH2	EMT	EZH2-EMT	(226,227)	
	stemness	EZH2 is the cell maker of stem cell	(45,228)	
	NED	1.Enz-AR-lncRNA-p21-EZH2-STAT3-NED	(54,55,81,82, 107, 116,141)	
		2.EZH2-NMyc-AR↓		
		2.CREB-EZH2-TSP1-NED		
REST	EMT	3.E2F1-RACGAP1-EZH2-NED		
		4.ADT-AR-EHF-EZH2-NED		
	stemness	1.KIT↓-REST-AR-SPINK1↓-EMT↓	(105,148,179)	
		2.REST-Twist1↓-EMT↓		
	NED	1.KIT↓-REST-AR-SPINK1↓-stemness	(105,148,179)	
		2.REST-CD44↓-stemness↓		
		1.KIT↓-REST-AR-SPINK1↓-NED↓		(56,57,58,85,105, 150,179,221)
		2.PI3K/AKT↓-REST↓-NED		
		3.IL-6-REST-NED		
		4.REST↓-ONECUT2-NED		
		5.REST↓-MAOA-NED		
		6.HP1α-AR and REST↓-NED		
E, Others				
Name	Role	Mechanisms	(Refs.)	
MUC1-C	EMT	MUC1-C-NF-κB p65-ZEB1-EMT	(143)	
	stemness	1.MUC1-C-E2F1-BAF-NOTCH1-HES1 and HEY1-EMT and stemness	(147,229)	
		2.MUC1-C-E2F1-BAF-NANOG-stemness		
	NED	3.MUC1-C-MYC-BRN2-SOX2-stemness	(110,229)	
		1.MUC1-C-AR↓, p53↓, RB↓, N-Myc and EZH2-NED		
2.MUC1-C-MYC-BRN2-SOX2-NED				
		3.MUC1-C-NF-κB p65-EZH2-NED		
Red text indicates core molecules. This table is not meant to be complete, as there are many other methods that simultaneously regulate EMT, stemness and NED. New ones are being discovered routinely. JAK/STAT, Janus Kinase/Signal Transducer and Activator of Transcription; EMT, Epithelial-to-Mesenchymal Transition; MMP, Matrix Metalloproteinase; IL-6, Interleukin 6; CCL3, C-C Motif Chemokine Ligand 3; TGF-β1, Transforming Growth Factor Beta 1; TP53, Tumor Protein p53; RB1, Retinoblastoma 1; NED, Neuroendocrine Differentiation; CHGA, Chromogranin A; ENO2, Enolase 2; PEDF, Pigment Epithelium-Derived Factor; RhoA, Ras Homolog Family Member A; NF-κB, Nuclear Factor Kappa B; mTOR, Mechanistic Target of Rapamycin; MAPK, Mitogen-Activated Protein Kinase; AR, Androgen Receptor; PI3K, Phosphoinositide 3-Kinase; AKT, Protein Kinase B; AMPK, AMP-Activated Protein Kinase; PTCH, Patched; SMO, Smoothened; PRKAR2B, Protein Kinase cAMP-Dependent Type II Regulatory Subunit Beta; DAB2IP, DAB2 Interacting Protein; ESM1, Endothelial Cell-Specific Molecule 1; TCF4, Transcription Factor 4; KIT, KIT Proto-Oncogene, Receptor Tyrosine Kinase; ROR2, Receptor Tyrosine Kinase Like Orphan Receptor 2; PKCδ, Protein Kinase C Delta; ERK, Extracellular Signal-Regulated Kinase; YY1, Yin Yang 1; FZD8, Frizzled Class Receptor 8; FYN, FYN Proto-Oncogene, Src Family Tyrosine Kinase; ADT, Androgen Deprivation Therapy; TCF7L1, Transcription Factor 7 Like 1; CXCR2, C-X-C Motif Chemokine Receptor 2; PCDH-PC, Protocadherin-PC; EZH2, Enhancer of Zeste Homolog 2; ALK, Anaplastic Lymphoma Kinase; N-Myc, N-Myc Proto-Oncogene; HIF-1α, Hypoxia-Inducible Factor 1 Alpha; SNAI1, Snail Family Transcriptional Repressor 1; DLL3, Delta Like Canonical Notch Ligand 3; Hes1, Hes Family BHLH Transcription Factor 1; Hey1, Hes Related Family BHLH Transcription Factor With YRPW Motif 1; Smad, Mothers Against Decapentaplegic Homolog; c-MYC, MYC Proto-Oncogene; SPOP, Speckle Type BTB/POZ Protein; CAF, Cancer-Associated Fibroblast; FOXC2, Forkhead Box C2; MAOA, Monoamine Oxidase A; LSD1, Lysine-Specific Demethylase 1; E2F, E2F Transcription Factor; KLF4, Kruppel-Like Factor 4; TCF3, Transcription Factor 3; PTEN, Phosphatase and Tensin Homolog; ZBTB46, Zinc Finger and BTB Domain Containing 46; PHF8, PHD Finger Protein 8; Siah2, Seven in Absentia Homolog 2; Jmjd1a, Jumonji Domain Containing 1A; SPINK1, Serine Peptidase Inhibitor Kazal Type 1; ASCL1, Achaete-Scute Family BHLH Transcription Factor 1; SRRM4, Serine/Arginine Repetitive Matrix 4; LIN28B, Lin-28 Homolog B; HMGA2, High Mobility Group AT-Hook 2; TRIM59, Tripartite Motif Containing 59; TRIB2, Tribbles Pseudokinase 2; BRN2, POU Class 3 Homeobox 2; TBX2, T-Box Transcription Factor 2; PEG10, Paternally Expressed 10; NCAM1, Neural Cell Adhesion Molecule 1; DLL1, Delta Like Canonical Notch Ligand 1; CEACAM5, Carcinoembryonic Antigen Related Cell Adhesion Molecule 5; TM4SF1, Transmembrane 4 L Six Family Member 1; SYP, Synaptophysin; lncRNA-p21, Long Non-Coding RNA p21; CREB, cAMP Response Element-Binding Protein; TSP1, Thrombospondin 1; RACGAP1, Rac GTPase Activating Protein 1; EHF, ETS Homologous Factor; CD44, CD44 Molecule; HP1α, Heterochromatin Protein 1 Alpha; BAF, BRG1/BRM-Associated Factor; HES1, Hes Family BHLH Transcription Factor 1; HEY1, Hes Related Family BHLH Transcription Factor With YRPW Motif 1.				

subunits: MUC1-N and MUC1-C subunit complex (143). The upregulation of MUC1-C in AR-dependent prostate cancer cells (LNCaP and C4-2) drives the progression of NEPC by inhibiting the p53 pathway and activating MYC (144,145). Additionally, MUC1-C promotes EZH2 transcription by binding to NF- κ B p65 at NF- κ B consensus sites within the EZH2 intron 1 enhancer region (145). MUC1-C inhibits AR signaling through multiple mechanisms, including post-transcriptional regulation (involving miR-135b-mediated downregulation of AR mRNA levels) and competitive inhibition (MUC1-C directly interacts with the AR DNA-binding domain through its cytoplasmic domain to form a complex with AR) (146).

The MUC1-C-activated MYC-BRN2 pathway is associated with the induction of the pluripotency factor SOX2 and NED markers (ASCL1, AURKA and SYP), while this pathway operates independently of EZH2, KLF4, or OCT4 expression (110). Compared with the MUC1-C-MYC-BRN2 pathway, the function of the MUC1-C-E2F1-BAF network serves as a parallel and unique driver of NED. Furthermore, in addition to driving NE, the MUC1-C-E2F1-BAF complex (comprising BRG1, ARID1A, BAF60a, BAF155 and BAF170)-NOTCH1-NANOG pathway also promotes EMT, stemness and the self-renewal ability of CSCs (147). MUC1-C can directly activate the inflammatory NF- κ B p65-ZEB1 pathway to induce EMT, which promotes cancer cell invasion and the acquisition of stem-like properties (143). SPINK1 induces EMT, stemness and cellular plasticity, playing a crucial role in maintaining the NE phenotype (105). Long-term loss of REST expression promotes EMT and stemness characteristics (148), while REST deficiency induces NED in prostate cancer cells by derepressing HOTAIR, MAOA and SPINK1 (149-151). The mechanisms of other pathways and molecules inducing EMT, stemness and NED are summarized in Table I.

7. Conclusion and future perspectives

With the widespread adoption of ADT, NEPC has emerged as an increasingly critical clinical challenge. The malignancy progressively escalates from prostate adenocarcinoma to mixed types and ultimately to NEPC, with a corresponding decline in patient prognosis. Activated signaling pathways are also involved in EMT, CSC characteristics/stemness, DNA damage response, metabolic reprogramming, hypoxia, ferroptosis and other biological processes, all of which are closely related to NED. Among these, the widespread activation of EMT and stemness has attracted particular attention. Identifying and validating core EMT/stem cell signaling pathways could lead to actionable biology that may be translated into therapeutic interventions. Therefore, the present study revisited the origins, mechanisms and the biological significance of EMT and stemness. The poor prognosis, high metastatic potential and resistance to radiotherapy, chemotherapy and endocrine therapy observed in NEPC are closely associated with EMT and stemness. Specifically, EMT confers high metastatic potential in NEPC, while stemness enables post-metastatic colonization, resistance to radiotherapy and chemotherapy and lineage plasticity. NED represents the ability of NEPC to resist castration. The NE

phenotype in prostate cancer does not necessarily imply high aggressiveness, as there are a number of pathological types with favorable prognosis. The NE molecular markers themselves hold limited clinical significance; rather, it is the activation of EMT and stemness during NED that endows NEPC with its distinct clinical characteristics. Studies have shown that mesenchymal and MSPC exhibits more advanced Gleason scores, pathological T stages and N stages compared with primary ARPC (69,152,153). A higher proportion of primary MSPC patients experience accelerated progression and die from the disease within 24 months of diagnosis (69).

EMT and stemness are critical characteristics of NEPC and represent essential aspects of studying highly aggressive prostate malignancies. In the future, EMT, stemness and NED should be considered as an integrated whole. Growing evidence indicates that conventional therapies frequently fail to eliminate cancer cells that have transitioned into a CSC state through the activation of EMT and NED programs, leading to CSC-mediated clinical relapse. The discovery and validation of potential core EMT/stemness signaling pathways in NEPC could uncover actionable biological mechanisms for therapeutic interventions.

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Authors' contributions

YFL and DLW participated in conceptualization and study design. YFL, SS and LDS conducted the investigation and data collection. SS and JW drafted the initial manuscript. CW, JH, and YL contributed to reviewing and editing. KH and YL were responsible for visualization. DW and XG supervised the study and finalized the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

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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