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Immunohistochemical Expression of NANOG in Cancer

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Abstract:

The homeobox domain transcription factor NANOG, a key regulator of embryonic development and cellular reprogramming, has been reported to be broadly expressed in human cancers. Functional studies have provided strong evidence that NANOG possesses protumorigenic attributes. In addition to promoting self-renewal and long-term proliferative potential of stem-like cancer cells, NANOG-mediated oncogenic reprogramming may underlie clinical manifestations of malignant disease. NANOG potentiates the molecular circuitry of tumorigenesis, and thus may represent a novel therapeutic target or biomarker for the diagnosis, prognosis, and treatment outcome of cancer.

Keywords: NANOG, Cancer, Tumor.

Introduction:

NANOG, a type of homeobox protein, is a transcription factor that contributes to embryonic stem cell pluripotency and self-renewal. It is additionally vital to stemness of embryonic cells through stifling the differentiation. NANOG, with OCT4, SOX-2, and KLF4, induces pluripotency in somatic cells **(1).**

NANOG is present in the pluripotent and developing germ cells in mammals. Its expression has also been reported in various malignancies, including breast, brain, colon, esophageal, prostate, and ovarian cancers **(2).**

NANOG structure

For the first time, NANOG was described in drosophila related to the NK homeobox genes, and it is detected in the short arm of chromosome 12 at 12p13.31 with four exons and three introns. NANOG promoter region includes five CpGdinucleotides subjected to DNA-methylation; these regions include an Oct3/4-Sox-2 binding motif that adjusts NANOG functions**(2).**

NANOG gene in the human chromosomal region possesses a tandem duplication. The two versions are around 97% the same and vary in their splicing. The second copy is named NanogP1 or Nanog2, which is an unprocessed pseudogene. In addition, ten Nanog pseudogenes known in the human genome are named from NanogP2 to NanogP11. Among them, only NanogP8 is translated into the protein and has an open reading frame located on chromosome 15q14**(3).**

In humans, the NANOG protein contains 305 amino acids with three functional domains. These domains include The N-terminal domain, a DNA-binding homeodomain, and the C-terminal domain, which are composed of 94, 60, and 151 amino acids, respectively **(4).**

Rather than using its homeodomain, NANOG dimerizes via its C-terminal domain. Nanog–Nanog homodimerization is required for interplay with other pluripotency network proteins, allowing them to operate more effectively in stem cell pluripotency promotion **(2).**

NANOG function and mechanism of action

NANOG is an important stem cell transcription factor that participates in normal cell development and tumorigenesis. NANOG regulates embryonic and fetal development and has a crucial role in the preimplantation development phase, with a progressive decrease during embryonic stem cell differentiation. After birth, a limited number of human tissues show a low level of expression in some cells in organs like the testis, ovary, and uterine glands, but most of the tissue is undetectable **(5).**

Re-expression of NANOG has been detected during carcinogenesis. Many studies identified that NANOG expression is already present in precancerous lesions, with rising levels in high-grade dysplasia. Therefore, it can be used as a diagnostic marker, distinguishing between true dysplasia and reactive lesions **(5).**

NANOG normally regulates embryonic stem cell (ESC) pluripotency and self-renewal via interactions with other transcription factors, like Sox-2 and Oct-4. These genes attach to octamer/sox elements in the NANOG promotional region, leading to the activation of NANOG transcription **(2).**

Various signaling pathways and miRNAs play an important role in the survival of CSCs by stabilizing NANOG. Therefore, targeting these signaling pathways or miRNAs that increase the level of expression of NANOG can be a targeted treatment strategy to eradicate CSCs. Also, targeting NANOG directly as a marker of CSCs can be a potential target for inhibiting their function. Theoretically, targeting of NANOG has several advantages, for example, NANOG is not expressed in somatic cells and is associated with several oncogenic pathways. Evidence suggests that using RNA-mediated interference (RNAi) techniques that directly target NANOG reduces tumor growth and development in CSCs **(6).**

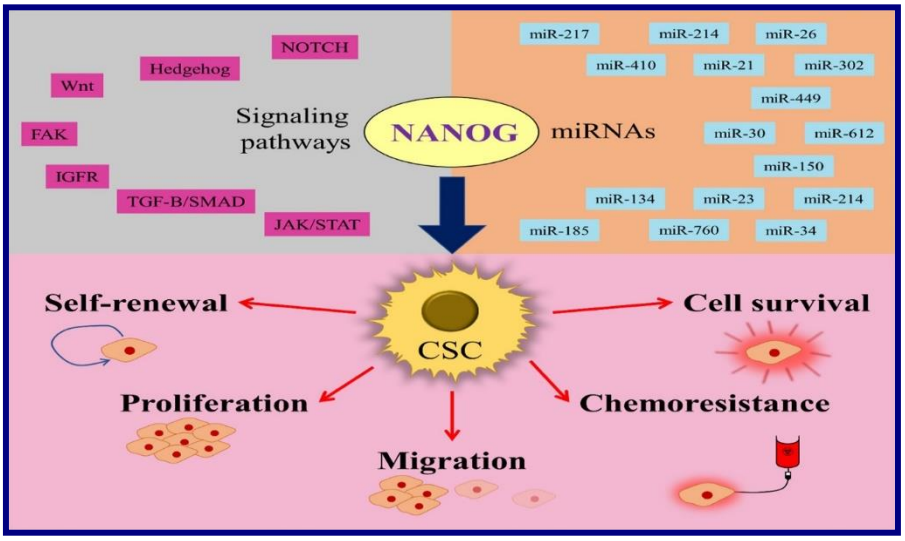

Figure (1): Various signaling pathways and miRNAs play an important role in the survival of CSCs by stabilizing NANOG **(6).**

Signaling pathways play an important role in controlling the function of normal and CSCs. Abnormal regulations of signaling pathways have been seen in many cancer cells that stimulate tumor progression and tumorigenesis. Also, their upregulation have been seen in CSCs that maintains the properties of stemness and increases selfrenewal, proliferation, invasion, and recurrence of the tumor. Furthermore, many signaling pathways such as JAK/STAT, Wnt/β-catenin, Notch, TGF-β, and Hedgehog pathways are positively correlated with increased NANOG expression in CSCs, leading to stemness characteristics **(7).**

Targeting these signaling pathways or proteins involved in these pathways with different techniques can reduce the population of CSCs. Thus, understanding the functional mechanism of signaling pathways in CSCs and their association with NANOG expression could be a promising strategy for cancer treatment **(8).**

miRNAs as key regulators are involved in controlling the expression of various genes. miRNAs in normal stem cells and CSCs regulate many biological processes, such as proliferation, differentiation, apoptosis, cancer initiation, embryonic development, and self-renewal **(7).**

miRNAs can act as a tumor suppressor and tumor promoter in many cancers. Studies have shown that miRNAs regulate the self-renewal and pluripotency of ESCs by regulating NANOG expression. Also, miRNAs maintain the properties of CSCs either directly or indirectly by regulating NANOG expression **(7).**

Post-translational modifications (PTMs) act as an “on-off switch” for regulate signaling cascades and play a key role in regulating cellular processes. In general, PTMs include phosphorylation, acetylation, methylation, formylation, PARylation, and ubiquitination **(6).**

PTMs occur in almost all proteins, especially transcription factors, and play a vital role in regulating proteins stability and function. Transcription factors such as NANOG, SOX2 and OCT4 regulate the function of stem cells and therefore a small change in their expression level induces tumorigenesis. Extensive studies show that the level of expression and stability and activity of NANOG are regulated through the PTMs. NANOG is one of the most important transcription factors that regulate ESCs and is subjected to a variety of PTMs, including ubiquitination, phosphorylation, and PARylation **(6).**

NANOG in cancer:

The embryonic stem cell self-renewal gene, NANOG, has been shown to be expressed in several tumor types and to regulate tumor development **(9).**

The upregulation of NANOG expression appears to lead cells to a process such as reprogramming but cannot maintain cells in a path that leads to a normal stemness state. As a result, by regulating several downstream signals, the cells are settled in a deviant path that participates in tumor formation **(2).**

NANOG overexpression has also been shown to promote proliferation and transformation of NIH3T3 cells. Together, these findings suggest that abnormal expression of NANOG in stem cells and tumor tissues plays a critical role in transformation, tumorigenicity, and metastasis **(10).**

An increased expression of NANOG has been detected in precancerous lesions (high-grade dysplasia), as well as in cancerous tissue **(11).** Cancer showing high NANOG expression is usually associated with high grade, advanced stage, worse overall survival, and resistance to treatment **(5).**

In Endometrial cancer

Endometrial CSC were described in primary endometrial carcinomas, identified by their clonogenic capacity and presence of genes associated with self-renewal. The resistance mechanisms related to the endometrial CSC are influenced by the presence of ABC efflux transporters, ALDH activity, resistance to DNA damage, autophagy, resistance to apoptosis, activation of developmental pathways and microenvironment stimuli **(9)**.

Transcription factors like OCT4, transcription factor 3 (TCF3), and SOX2 that regulate NANOG expression were found in endometrial CSCs and related to the potential ability for self-renewal **(5).**

A minority of studies investigated NANOG in EC.

Liver cancer

Multiple subtypes of hepatocellular carcinoma have liver cancer stem cells, contributing to tumor initiation, metastasis, and development. To maintain self-renewal and pluripotency, liver CSCs must control multiple signaling paths, including EpCAM, Wnt/β-catenin, Sonic Hedgehog, and Notch. Also, lately, identified liver CSCs markers, including OCT4, SOX-2, and NANOG in the human hepatoma cell line, play an imperative role in keeping up stemness **(2).**

Colorectal cancer

Colorectal cancer includes a heterogeneous population of cancer cells like CSCs that can induce self-renewal. The pathogenesis of colorectal cancer may be developed through CSC **(12).**

Also, researches indicated that NANOG acts as a tumor marker for colorectal cancer subjects and is associated with clinical and pathological features **(13).** The upregulation of NANOG in colorectal CSCs was positively correlated with the regulation of c-JUN **(2).**

Brain cancer

Vasefifar et al., (2) reported elevated NANOG in GBM6, GBM10, and GBM14 xenograft cell lines, and its expression in the early generation (15 passages) is more than afterward generation (24 passages) xenografts. Then, limited passaging maintains the brain tumor stem cell's multipotent features. Glioblastoma patients with overexpression of CD24 and NANOG manifest poor survival.

Prostate cancer

Studies have shown that prostate cancer cells acquire stem-like properties through the expression of NANOG, particularly in stable and accumulated conditions. Phosphorylation of NANOG is essential to maintain NANOG stability, thereby enhancing tumorigenic properties**(14).**

In prostate cancer, miR-218 overexpression downregulates NANOG, OCT4, vimentin, and CD44 expression level. Thus, this miR inhibits tumoral migration and EMT in prostate cancer cells **(15).**

Furthermore, NANOG can regulate the HDAC1, histone deacetylases in protein and transcriptional level. NANOG phosphorylates and inactivates CHFR (an E3 ubiquitin ligase) via the AKT signaling pathway. This accumulates HDAC1 instead of its proteasomal degradation. In line with this, accumulated HDAC1 accounts for metastasis in various cancers, and the AKT signaling inhibition degrades HDAC1 **(16).** In CSCs ERK1/2-β-catenin signaling upregulates NANOG and promotes metastasis, tumorigenesis, and radiotherapy resistance. Moreover, the NANOG-ERK1/2 signaling pathway enhances N-cadherin and Snail, which further metastasis, and tumor progression **(17).**

Ovarian cancer

NANOG, as a stemness marker, is critical for preserving the features of ovarian cancer. In line with that, IHC assessment in ovarian cancer patients shows that high levels of NANOG were directly related to the pathological grade and tumor stage. NANOG expression is correlated to a poor prognosis and shorter overall survival, suggesting that it might be a potential biomarker for ovarian carcinoma patients' responsiveness to paclitaxel-based chemotherapy **(2).**

Leukemia

NANOG is abundant in CD34+CD38- leukemia CSCs. NANOG via insulin-like growth factor receptor (IGF1R) signaling induces proliferation and suppresses cell cycle arrest and cell apoptosis. T-cell acute lymphoblastic leukemia (T-ALL) cells express NANOG. Its knockdown via siRNA hampers CSCs' properties and causes apoptosis and cell cycle arrest via p53 in leukemic cells **(2).**

Pancreatic cancer

Co-immunoprecipitation experiments in SW1990 cells showed that SPOP interacted with the stem-cell marker NANOG, and this interaction has recently been shown to play a critical role in regulating progression of pancreatic cancer **(18).**

Tan et al., (18) showed that, in one patient with pancreatic cancer, the expression of a truncated form of SPOP (p.Q360*) lacking the nuclear localization signal led to nuclear accumulation of NANOG, which promoted growth and metastasis of pancreatic cancer cells.

Breast cancer

Since CSCs are resistant to chemotherapy; then tumor recurrence occurs after treatment due to the existence of CSC in breast tumor tissue. Therefore, targeting CSC can be an effective treatment. This targeting includes CSC surface proteins, cell cycle quiescence, molecular pathways, and microRNA signaling. NANOG activity is affected by the HA-CD44 signaling pathway in breast cancer cells **(2).**

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