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Regulation of NANOG in cancer cells

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Abstract

As one of the key pluripotency transcription factors, NANOG plays a critical role in maintaining the self-renewal and pluripotency in normal embryonic stem cells. Recent data indicate that NANOG is expressed in a variety of cancers and its expression correlates with poor survival in cancer patients. Of interest, many studies suggest that NANOG enhances the defined characteristics of cancer stem cells and may thus function as an oncogene to promote carcinogenesis. Therefore, NANOG expression determines the cell fate not only in pluripotent cells but also in cancer cells. Although the regulation of NANOG in normal embryonic stem cells is reasonably well understood, the regulation of NANOG in cancer cells has only emerged recently. The current review provides a most updated summary on how NANOG expression is regulated during tumor development and progression.

Keywords

NANOG; NANOGP8; cancer stem cells; tumor development

INTRODUCTION

Recent studies indicate that most human tumors harbor a population of cancer cells termed cancer stem cells (CSCs) that possess biological properties characteristic of normal stem cells, i.e., self-renewal and differentiation [1]. CSCs are thought to be immortal being able to persist in tumors and to contribute to relapse and metastasis [2, 3]. As CSCs can potentially arise from oncogenic reprogramming, identification of stem cell-related and self-renewal molecules (transcription factors, cell surface proteins, stemness genes, and microRNAs) responsible for the manifestation of stem cell properties of somatic cancer cells is a critical question and can in turn lead to identification of novel therapeutic targets for cancer. Recently accumulated evidence suggests that NANOG is a crucial factor that can confer

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cancer cells certain CSC properties such as self-renewal, tumorigenicity, metastasis, and drug-resistance.

NANOG is a divergent homeobox domain protein first discovered in embryonic stem cells (ESCs) with canonical functions in the transcriptional regulation of self-renewal and pluripotency [4, 5]. Together with SOX2 and OCT4, NANOG plays a key role in maintaining the properties of ESCs [6, 7]. Through forming a transcriptional network, the three key factors generally function together to control the expression of a whole set of pluripotent-related genes and establish the pluripotency of ESCs [4, 5]. NANOG is highly expressed in pluripotent cells such as ESCs, and EG (embryonic germ) and EC (embryonal carcinoma) cells, and its expression is downregulated upon differentiation [4]. Overexpression of Nanog^{*} protein not only maintains the pluripotency of mouse ESCs in the absence of extrinsic factor, leukemia inhibitory factor (LIF) [4, 8], but also promotes human ESC growth in feeder-free conditions [9]. Moreover, ectopic NANOG expression can improve reprogramming in a cell-division-rate-independent manner [10]. In contrast, downregulation of NANOG induces both mouse and human ESC differentiation [9, 11]. Therefore, NANOG protein level determines the fate of pluripotent cells.

Although silenced in normal somatic cells, aberrant expression of NANOG has been reported in many types of human cancers, including carcinomas of the brain, breast, cervix, colon, gastric, head and neck, liver, lung, kidney, oral cavity, ovary, pancreas, prostate, and other organs [12–30]. Importantly, the expression levels of NANOG are often positively correlated with treatment resistance and poor survival of cancer patients. Various studies have shown that upregulation of NANOG expression enhances the tumorigenicity both *in vivo* and *in vitro* whereas repression or ablation of NANOG inhibits tumor initiation. Thus, NANOG expression is linked to tumor progression, therapeutic resistance, relapse and metastasis. In this review, we focus our discussion on how NANOG expression could be regulated during various tumorigenic processes.

Regulation of NANOG during tumor progression

Human ESC NANOG arises from *NANOG1*, composed of four exons, three introns, and a 915 bp open reading frame (ORF), located on chromosome 12. As is common for genes expressed during embryogenesis, there exist numerous copies of *NANOG* scattered throughout the genome, including one tandem duplication (*NANOG2*, also called *NANOGP1*) and ten intronless retrotransposed paralogs, identified as *NANOGP2* to *NANOGP11* [31]

The majority of NANOG retrogene variants are considered pseudogenes, as these are degenerate copies with various defects such as deletions, frame shifts, premature stop codons, etc. Only *NANOGP8*, located on chromosome 15, has a complete ORF and can encode a protein nearly identical to that encoded by *NANOG1*, with the exception of two conserved amino acid substitutions (Ala16 to Glu, and Gln253 to His) [28, 31, 32]. To date,

^{*}Note: In this paper, NANOG is used to refer to human protein whereas Nanog to mouse protein and Nanog protein/mRNA/gene in general.

several pieces of evidence suggest that NANOGP8 exerts divergent biological functions in prostate cancer cells [28, 33, 34].

In ESCs, regulation of NANOG expression has been extensively investigated since NANOG was discovered, and many proteins have been reported to be capable of modulating the expression of NANOG. For example, TCF3 and p53 negatively regulate NANOG expression by binding to the promoter of *NANOG* whereas LIF and BMP signaling and their downstream effectors STAT3 and T may also be involved in NANOG regulation [35–37]. Among the NANOG-regulating proteins, SOX2 and OCT4 play a particularly important role via a composite Oct4/Sox2 motif localized ~180 bp upstream of the transcription start site of the *NANOG* promoter [38, 39].

In contrast to our knowledge on NANOG regulation in ESCs, we have just begun to understand how NANOG might be regulated at the molecular level in cancer cells. Here, we discuss the evidence for regulation of NANOG expression in cancer cells by active STAT3, p53, HH signaling, microRNAs, hypoxia, and various post-translational modifications (Figure 1).

1. Regulation of NANOG by STAT3

In ESCs, STAT3 is the major effector of the LIF pathway that is required for the maintenance of ESC stemness. LIF-induced phosphorylation of STAT3 (pSTAT3, the active form) directly binds to the enhancer of the *Nanog* gene, hence upregulating the transcription of *Nanog* in mouse ES cells. Interestingly, E-Cadherin in mouse ES cells greatly promotes Nanog expression at both mRNA and protein levels possibly involving pSTAT3-induced upregulation of Nanog transcription [40].

Aberrant STAT3 signaling, i.e., the constitutively active STAT3 has been implicated in multiple tumor systems and tumorigenic processes. For example, in hepatocellular carcinoma (HCC), pSTAT3 has been shown to positively regulate NANOG expression. In a human HCC cell line HepG2, the Hepatitis C virus (HCV) core exhibited the ability to upregulate the expression of NANOG, leading to cell proliferation. In this cell model, HCV enhanced the activity of STAT3, and the pSTAT3 then directly bound the regulatory regions of *NANOG* to enhance the expression of NANOG [41] (Figure 1). In breast cancer cells, NANOG formed a complex with STAT3, which then translocated into the nucleus to regulate their common target genes [15].

2. Regulation of NANOG by p53 and Hedgehog pathways

The transcription factor p53 represents one of the most important tumor suppressors due to its ability to induce cell apoptosis and senescence, promote DNA repair, inhibit epithelial to mesenchymal transition (EMT), and mediate many other processes [42–44]. Hedgehog (HH) and its downstream transcription factors Gli1, Gli2 (HH-GLI), are also important regulators that promote the self-renewal of ESCs and postnatal neuronal stem cells (NSCs) during early embryonic and nervous system development [45, 46]. In mouse ESCs, p53 directly binds the promoter of *Nanog* and negatively regulates its transcription in response to DNA damage [36]. Thus, loss of p53 enhances the reprogramming efficiency leading to increased

generation of induced pluripotent stem (iPS) cells. Two studies have shown that HH-GLI signaling pathway and p53 directly regulate the expression of NANOG in neural stem cells from brain tissues and brain tumors [12,13]. Po et al detected high levels of NANOG protein in stem cells from cerebellum and medulloblastoma and identified that Gli1 and Gli2 interacted with the promoter region of *NANOG* and activated the transcription of *NANOG* gene [12]. They also demonstrated that the HH, in cooperation with loss of p53, positively modulated NANOG expression. Meanwhile, Zbinden et al reported that NANOG, mainly NANOGP8, is highly expressed in the glioblastoma multiforme (GBM) and NANOG/P8 controlled the proliferation of GBM stem cells and contributed to the tumorigenicity of GBM [13]. They further demonstrated that GLI, and NANOG formed a positive loop by regulating each other, and the positive loop was suppressed by p53. These findings led the authors to hypothesize that the functional network of HH-GLI-NANOG-p53 plays a critical role in regulating glioma stem cells [13] (Figure 1).

3. Regulation of NANOG by MicroRNAs

MicroRNAs (miRNAs) are small, nonprotein-coding RNAs, roughly 19–24 nucleotides in length [47, 48]. As endogenous regulators of gene expression, miRNAs induce translational repression or transcript degradation by binding to the complementary sites in the 3'-untranslated region (3'-UTR) of their target genes, thereby playing a crucial role in diverse physiologic processes such as proliferation, differentiation, apoptosis, and stem cell maintenance [49–51]. Dysregulation in miRNA expression or functions is involved in various human cancers, indicating that miRNAs have an important impact on the tumorigenesis. For example, our group has reported that microRNA-34a (miR-34a) acts as a tumor suppressor to restrict clonogenicity, sphere formation, tumor growth and metastasis of prostate CSCs by directly repressing the expression of CD44 [52]. Newly accumulated evidence suggests that miRNAs also target NANOG directly or indirectly to modulate tumorigenic processes.

miR-134—miR-134 is a brain-enriched microRNA essential for vertebrate central nervous system development [53, 54]. miR-134 expression is dramatically reduced in glioma tissues compared with normal brain tissues and the reduction of miR-134 is strongly associated with aggressive progression and poor prognosis in gliomas, indicating that miR-134 serves as a tumor-suppressive miRNA in brain tumors [55]. The regulation of Nanog expression by miR-134 was first reported in mouse ESCs [53]. Recently, Niu et al provided direct evidence to support miR-134 functioning as a tumor suppressor in human gliomas by targeting NANOG [56]. They found that the miR-134 level was not only significantly lower in clinical glioma samples and glioblastoma cell line U87 compared to normal brain tissues, but was also greatly reduced in grade III and IV gliomas compared to grade I and II tumors. miR-134 promoted apoptosis, impaired the invasiveness and migration capability, and inhibited proliferation of U87 cells. Importantly, overexpression of miR-134 in U87 cells directly targeted the *NANOG* gene to reduce its mRNA and protein levels [56].

miR-128—miR-128 is also a brain-enriched microRNA with developmental-specific expression patterns, mainly in mature, differentiated neurons rather than in astrocytes [57]. miR-128 has been observed to be downregulated in many tumors including glioblastoma,

medulloblastoma [58, 59], breast cancer [60], prostate cancer [61], and ovarian cancer [62], suggesting that miR128 is also a tumor-suppressive miRNA. Recently, our group reported that overexpression of miR-128 inhibited prostate cancer cell proliferation, invasion, and clonogenic and sphere-formation in vitro, and suppressed tumor regeneration in vivo [63]. We also showed that the tumor-suppressive functions of miR128 were related to its ability to directly target several 'stemness' genes including NANOG and BMI-1 [63].

miR-126 and miR-149—It was recently reported that NANOG, SOX2, and OCT4 in prostate cancer cell lines were regulated by miR-126 and miR-149. With quantitative RT-PCR, the authors observed that inhibition of miR-126 or miR-149 by their inhibitors significantly elevated the mRNA levels of NANOG, SOX2, and OCT4 [64].

miR-214—miR-214 is considered an oncogenic miRNA based on the observations that not only is its aberrant expression detected in multiple human cancers [65–67] but also elevated miR-214 levels are positively associated with chemoresistance and metastasis [66, 67]. Studying the functions of miR-214 in ovarian cancer cells, Xu et al provided an example of how miRNAs could indirectly regulate NANOG expression [65]. They found that enforced miR-214 expression led to enhanced NANOG expression and characteristics of ovarian CSCs (OCSCs). In contrast, miR-214 knockdown decreased NANOG expression and OCSC properties. Mechanistic studies showed that miR-214 directly interacted with the 3'-UTR of p53 to repress p53, which, in turn, led to increased NANOG expression. Elevation of NANOG expression by miR-214 was p53-dependent. These results together indicate that miR-214 regulates NANOG expression via targeting p53 [65] (Figure 1).

4. Regulation of NANOG by hypoxia

Hypoxia, defined as oxygen deprivation, is a common feature of the solid tumor microenvironment and often occurs in a pathophysiological condition where the consumption of oxygen exceeds the blood supply, leading to local tumor regions with very low level of oxygen. Hypoxia often exists in the inner part of solid tumors, creating an environment beneficial to the growth of undifferentiated tumor cells [33]. Therefore, hypoxia is considered as a critical component of the CSC niche and plays an important role in the maintenance of CSC behavior and properties, including self-renewal, differentiation, invasion, metastasis, therapeutic resistance, and genetic instability. The response to hypoxia by tumor cells is mainly mediated by the family of hypoxia-inducible factor (HIF) transcription factors.

Recent evidence shows that hypoxia promotes the expression of ESC markers such as NANOG, OCT4, SOX2, KLF4 and cMYC in several tumor types. For example, Ruohola-Baker' group used RT-PCR strategy to investigate the gene expression signature of 11 different cancer cell lines under hypoxic conditions (2% O₂). The cell lines included prostate, brain, kidney, cervix, lung, colon, liver, and breast tumors. They discovered that hypoxia upregulated the expression of reprogramming inducing genes: NANOG, OCT4, SOX2, KLF4, cMYC, and the miR-302 cluster, and verified that the genes unregulated in cancer cell lines were HIF-dependent. By employing immunohistochemical staining, they further observed a strong correlation between NANOG-, OCT4-, and HIF-positive region in

primary prostate tumor samples and the significantly higher expression of NANOG and OCT4 in high-grade prostate cancer [68].

Similarly, Wu et al reported that hypoxia treatment of laryngeal cancer cell lines significantly expanded G0/G1 stage (a fundamental feature of stem cells) and increased the CD133⁺ CSC subpopulation [69]. They also found that hypoxia increased the mRNA levels of stem cell genes *NANOG*, *OCT4*, and *SOX2*, as well as their protein levels [69]. Consistent with the results in laryngeal cancer cells, another group independently reported that hypoxia exhibited similar effects on extending the G0/G1 stage and increasing the CD44⁺ and ABCG2⁺ cell populations in prostate cancer cells lines, PC3 and Du145 [33]. Upon hypoxia treatment (1% O₂), the expression of NANOG, OCT4, HIF-1 α , and HIF-2 α was greatly elevated at both mRNA and protein levels. Moreover, up-regulated *NANOG* mRNA expression by hypoxia was predominately derived from retrogene NANOGP8 locus [33]. Hypoxia has also been shown to selectively enhance the expression of NANOG in human non-small cell lung carcinoma cells, leading to resistance to CTL-mediated lysis [70].

Taken together, the above findings indicate that hypoxia, via HIF, induces the expression of NANOG (Figure 1) and other stem cell makers (OCT4, SOX2, MYC, etc.) and support the notion that hypoxia promotes cancer progression via, at least partly, regulating the CSC properties.

5. Regulation of NANOG by post-translational modifications

Post-translational modifications (PTMs) occur on nearly all proteins, particularly on transcription factors, and represent a powerful approach to regulate the stability and functions of proteins. PTMs can function as an “on-off switch” to control signaling cascades and play the critical roles in regulating multiple cellular functions such as transcriptional activity, subcellular localization, protein folding, and protein stability, etc. In general, PTMs include amino acid modifications (phosphorylation, acetylation, methylation, amidation, formylation, etc) and group attachment (e.g., ubiquitination, sumoylation, and neddylation).

As one of the master regulators in ESCs, NANOG is subject to multiple types of PTMs and the modifications confer NANOG with diverse functions. Because NANOG protein harbors numerous serine (Ser), threonine (Thr), and tyrosine (Tyr) residues, it is natural to hypothesize that NANOG might be modified by phosphorylation (Figure 1). Indeed, in mouse ESCs, Nanog is phosphorylated at Ser52, Ser65, Ser71 and Thr-287 by an unidentified kinase. This phosphorylation facilitates the binding between Nanog and the prolyl isomerase Pin1, and hence improves the stability of Nanog by preventing Nanog degradation from ubiquitin-mediated degradation [71]. In support, by developing a multiplexed assay for kinase specificity (MAKS), Brumbaugh *et al* reported that NANOG contains 11 phosphorylation sites including Ser52, Ser65, Ser71 and that ERK2 directly phosphorylates NANOG at Ser52 *in vitro* [72]. However, work from Dong’s group indicated that phosphorylation of Nanog in mouse ESCs at Ser52, Ser71, and Ser78 (not Ser65), by ERK1 rather than ERK2, inhibited NANOG transactivation, induced cell differentiation, and reduced Nanog stability through proteomic degradation [73]. In fact, inhibition of ERK1 activity promoted Nanog transactivation and increased the protein levels. Their findings suggest that the Nanog protein level is modulated through ERK1 phosphorylation, and

ERK1 signaling negatively regulates Nanog activity in maintenance of ESC pluripotency [73].

In human head and neck squamous cell carcinoma (HNSCC) cells, Pan's group found that NANOG was phosphorylated by protein kinase C ϵ (PKC ϵ) at multiple sites and the phosphorylation at Thr200 and Thr280 enhanced NANOG activity, promoted NANOG protein stability, increased NANOG homodimerization, and regulated Bmi1 activity by binding the promoter of BMI1, and thereby induced tumorigenesis [74]. Accordingly, forced expression of phosphorylation-insensitive T200A or T280A mutant NANOG impaired its capacity to promote cell proliferation, colony formation, invasion, migration and the CSC expansion. Their results illustrate that phosphorylation of NANOG at certain sites is required for sustaining NANOG stability, dimerization, and regulating BMI1 and thus promoting tumorigenic properties in HNSCC cells [74]. Unexpectedly, another group presented opposite results and conclusions for effects of PKC on NANOG [75]. The authors investigated the relationship between PKC activity and NANOG expression in several human cancer cell lines including nasopharyngeal carcinoma NPC-076, hepatocellular carcinoma (HepG2 and Hep3B), bladder carcinoma (HT1376 and T24), colorectal cancer (SW620) and embryonal carcinoma cells (NT2/D1 and NCCIT). They observed that either inhibition or knockdown PKC, particularly PKC α and PKC δ , dramatically promoted NANOG expression, and PKC inhibitors enhanced *NANOG* promoter activity, whereas activation of PKC by phorbol-12-myristate-13-acetate (PMA) suppressed NANOG and its target genes expression [75]. The induction of NANOG expression by PKC inhibitors required Octamer–Sox composite element. The reasons behind the discrepancy of the differential PKC effects on NANOG expression in the two studies [74,75] need further investigation. Another study reported that NANOG not only promoted the activity and expression of focal adhesion kinase (FAK) by binding the promoter region of *FAK*, but also interacted with FAK protein, which, in turn, phosphorylated NANOG at Tyr 35 and Tyr174 in a dose dependent manner [76]. The phosphorylation of NANOG by FAK significantly promoted cell invasion of HEK293 and human colon cancer cells SW480 and SW620 [76].

Besides phosphorylation (Figure 1), NANOG stability in ESCs is also affected by ubiquitination through its PEST motif [77]. Additionally, NANOG expression is negatively regulated by sumoylation in pluripotent cells [78]. Whether similar modifications in NANOG exist in cancer cells remains unclear.

CONCLUSIONS AND PERSPECTIVES

There is no doubt that many other molecules may also have the capacity to regulate NANOG expression. For example, BMI1, a transcriptional repressor belonging to the Polycomb group of proteins, has been shown to significantly enhance NANOG expression in breast cancer cells [79]. In prostate cancer cells, androgen receptor (AR) has been reported to directly bind to the *NANOG* promoter and hence upregulate NANOG expression at both mRNA and protein level [80].

Regarding the regulation of NANOG in cancer cells, most current studies focus on the transcription, mainly based on the regulation of *NANOG1* gene. The most popular mode of

regulation is that the effectors (e.g., p53, p-STAT3, HIF) directly bind to the promoter region of *NANOG1* and consequently modulates NANOG expression (Figure 1). However, there is strong evidence that *NANOG* mRNA transcripts in a variety of cancer cell lines and tumor samples are predominantly derived from the NANOGP8 locus, whereas *NANOG1* is highly expressed only in pluripotent cells. Therefore, several questions and caveats are associated with many of these studies. **First**, human *NANOG1* and *NANOGP8* genes are located on chromosomes 12 and 15, respectively, and the two genes possess different promoter regions. Theoretically, the regulatory mechanisms for their expression in cancer cells should be distinct. If the majority of *NANOG* mRNA species are derived from the retrogene NANOGP8 locus, how could the mechanisms that engage the 5'-regulatory sequences of *NANOG1* operate to regulate NANOGP8 expression? In fact, only very few papers presented some evidence relating to the regulation of NANOGP8 in cancer cells (Figure 2). In GBM CSCs, Zbinden et al have demonstrated that NANOGP8 and GLI1 positively regulate each other and form a protein complex, which is antagonized by p53; consequently, NANOGP8, Gli1, and p53 form a functional network to modulate GBM CSCs [13] (Figure 2). Ma et al have observed that hypoxia enhances the expression of NANOGP8 but not *NANOG1* in prostate cancer cells maintained under hypoxic conditions [33], presumably via HIF1-mediated transactivation (Figure 2). However, both papers did not provide the detailed molecular mechanisms as to how the *NANOGP8* retrogene might be regulated in the two types of cancer cells. Through TRANSFAC analyses of the NANOGP8 promoter, we have found several putative NANOGP8 promoter-binding factors include SP1, MYC (c-MYC), TCF, and ETS (Jeter et al, unpublished data), suggesting that these factors might be involved regulating NANOGP8 expression in cancer cells. Clearly, more detailed and extensive investigations on how NANOGP8 expression in somatic cancer cells are needed.

Second, because microRNAs function mainly through binding to the complementary sequences in the 3'-UTR of their target transcripts, and because 3'-UTR's of *NANOG1* and *NANOGP8* are nearly identical, it is reasonable to speculate that those microRNAs that function as either tumor suppressive or oncogenic to target *NANOG1* transcripts should have similar impacts on the expression of NANOGP8. **Third**, as the two conserved amino acid differences between *NANOG1* protein and *NANOGP8* protein occur at the residue 16 (Ala to Glu) and residue 253 (Gln to His), and the two amino acid changes do not involve Ser, Thr, Tyr, and Lys (i.e., potential phosphorylation, ubiquitination and sumoylation sites), it is tempting to speculate that signaling pathways that result in PTMs on *NANOG1* should exert similar effects on NANOGP8.

NANOG expression has been reported to be elevated in a variety of cancers, and NANOG expression levels seem to positively correlate with the malignancy and patient survival, implicating NANOG as an oncogenic factor in cancer development. Because NANOG is not expressed in most normal adult tissues, NANOG and its related signaling pathways might represent novel targets for therapeutic development. Thus, understanding the regulation of NANOG during cancer progression will be of significance in developing new strategies to battle against cancer.

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REFERENCES

1. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer*. 2008; 8:755–768. [PubMed: 18784658]
2. Zhang S, Balch C, Chan MW, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res*. 2008; 68:4311–4320. [PubMed: 18519691]
3. Alvero AB, Chen R, Fu HH, et al. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. *Cell Cycle*. 2009; 8:158–166. [PubMed: 19158483]
4. Chambers I, Colby D, Robertson M, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell*. 2003; 113:643–655. [PubMed: 12787505]
5. Mitsui K, Tokuzawa Y, Itoh H, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell*. 2003; 113:631–642. [PubMed: 12787504]
6. Boiani M, Scholer HR. Regulatory networks in embryo-derived pluripotent stem cells. *Nat Rev Mol Cell Biol*. 2005; 6:872–884. [PubMed: 16227977]
7. Nichols J, Zevnik B, Anastassiadis K, et al. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell*. 1998; 95:379–391. [PubMed: 9814708]
8. Pei D. Regulation of pluripotency and reprogramming by transcription factors. *J Biol Chem*. 2009; 284:3365–3369. [PubMed: 18819918]
9. Darr H, Mayshar Y, Benvenisty N. Overexpression of NANOG in human ES cells enables feeder-free growth while inducing primitive ectoderm features. *Development*. 2006; 133:1193–1201. [PubMed: 16501172]
10. Hanna J, Saha K, Pando B, et al. Direct cell reprogramming is a stochastic process amenable to acceleration. *Nature*. 2009; 462:595–601. [PubMed: 19898493]
11. Hyslop L, Stojkovic M, Armstrong L, et al. Downregulation of NANOG induces differentiation of human embryonic stem cells to extraembryonic lineages. *Stem Cells*. 2005; 23:1035–1043. [PubMed: 15983365]
12. Po A, Ferretti E, Miele E, et al. Hedgehog controls neural stem cells through p53-independent regulation of Nanog. *EMBO J*. 2010; 29:2646–2658. [PubMed: 20581804]
13. Zbinden M, Duquet A, Lorente-Trigos A, et al. NANOG regulates glioma stem cells and is essential in vivo acting in a cross-functional network with GLI1 and p53. *EMBO J*. 2010; 29:2659–2674. [PubMed: 20581802]
14. Nagata T, Shimada Y, Sekine S, et al. Prognostic significance of NANOG and KLF4 for breast cancer. *Breast Cancer*. 2014; 21:96–101. [PubMed: 22528804]
15. Bourguignon LY, Peyrollier K, Xia W, Gilad E. Hyaluronan-CD44 interaction activates stem cell marker Nanog, Stat-3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells. *J Biol Chem*. 2008; 283:17635–17651. [PubMed: 18441325]
16. Ye F, Zhou C, Cheng Q, Shen J, Chen H. Stem-cell-abundant proteins Nanog, Nucleostemin and Musashi1 are highly expressed in malignant cervical epithelial cells. *BMC Cancer*. 2008; 8:108. [PubMed: 18419830]
17. Lin T, Ding YQ, Li JM. Overexpression of Nanog protein is associated with poor prognosis in gastric adenocarcinoma. *Med Oncol*. 2012; 29:878–885. [PubMed: 21336986]
18. Meng HM, Zheng P, Wang XY, et al. Overexpression of nanog predicts tumor progression and poor prognosis in colorectal cancer. *Cancer Biol Ther*. 2010; 9:295–302. [PubMed: 20026903]
19. Zhang J, Espinoza LA, Kinders RJ, et al. NANOG modulates stemness in human colorectal cancer. *Oncogene*. 2013; 32:4397–4405. [PubMed: 23085761]

20. Yu CC, Chen YW, Chiou GY, et al. MicroRNA let-7a represses chemoresistance and tumorigenicity in head and neck cancer via stem-like properties ablation. *Oral Oncol.* 2011; 47:202–210. [PubMed: 21292542]
21. Shan J, Shen J, Liu L, et al. Nanog regulates self-renewal of cancer stem cells through the insulin-like growth factor pathway in human hepatocellular carcinoma. *Hepatol.* 2012; 56:1004–1014.
22. Chiou SH, Wang ML, Chou YT, et al. Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation. *Cancer Res.* 2010; 70:10433–10444. [PubMed: 21159654]
23. Li XQ, Yang XL, Zhang G, et al. Nuclear beta-catenin accumulation is associated with increased expression of Nanog protein and predicts poor prognosis of non-small cell lung cancer. *J Transl Med.* 2013; 11:114. [PubMed: 23648139]
24. Bussolati B, Bruno S, Grange C, Ferrando U, Camussi G. Identification of a tumor-initiating stem cell population in human renal carcinomas. *FASEB J.* 2008; 22:3696–3705. [PubMed: 18614581]
25. Chiou SH, Yu CC, Huang CY, et al. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. *Clin Cancer Res.* 2008; 14:4085–4095. [PubMed: 18593985]
26. Lee M, Nam EJ, Kim SW, Kim S, Kim JH, Kim YT. Prognostic impact of the cancer stem cell-related marker NANOG in ovarian serous carcinoma. *Int J Gynecol Cancer.* 2012; 22:1489–1496. [PubMed: 23095773]
27. Wen J, Park JY, Park KH, et al. Oct4 and Nanog expression is associated with early stages of pancreatic carcinogenesis. *Pancreas.* 2010; 39:622–626. [PubMed: 20173672]
28. Jeter CR, Badeaux M, Choy G, et al. Functional evidence that the self-renewal gene NANOG regulates human tumor development. *Stem Cells.* 2009; 27:993–1005. [PubMed: 19415763]
29. Jeter CR, Liu B, Liu X, et al. NANOG promotes cancer stem cell characteristics and prostate cancer resistance to androgen deprivation. *Oncogene.* 2011; 30:3833–3845. [PubMed: 21499299]
30. Hart AH, Hartley L, Parker K, et al. The pluripotency homeobox gene NANOG is expressed in human germ cell tumors. *Cancer.* 2005; 104:2092–2098. [PubMed: 16206293]
31. Booth HA, Holland PW. Eleven daughters of NANOG. *Genomics.* 2004; 84:229–238. [PubMed: 15233988]
32. Zhang J, Wang X, Li M, et al. NANOGP8 is a retrogene expressed in cancers. *FEBS J.* 2006; 273:1723–1730. [PubMed: 16623708]
33. Ma Y, Liang D, Liu J, et al. Prostate cancer cell lines under hypoxia exhibit greater stem-like properties. *PLoS One.* 2011; 6:e29170. [PubMed: 22216200]
34. Zhang K, Fowler M, Glass J, Yin H. Activated 5'flanking region of NANOGP8 in a self-renewal environment is associated with increased sphere formation and tumor growth of prostate cancer cells. *Prostate.* 2014; 74:381–394. [PubMed: 24318967]
35. Pereira L, Yi F, Merrill BJ. Repression of Nanog gene transcription by Tcf3 limits embryonic stem cell self-renewal. *Mol Cell Biol.* 2006; 26:7479–7491. [PubMed: 16894029]
36. Lin T, Chao C, Saito S, et al. p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat Cell Biol.* 2005; 7:165–171. [PubMed: 15619621]
37. Suzuki A, Raya A, Kawakami Y, et al. Nanog binds to Smad1 and blocks bone morphogenetic protein-induced differentiation of embryonic stem cells. *Proc Natl Acad Sci USA.* 2006; 103:10294–10299. [PubMed: 16801560]
38. Rodda DJ, Chew JL, Lim LH, et al. Transcriptional regulation of nanog by OCT4 and SOX2. *J Biol Chem.* 2005; 280:24731–24737. [PubMed: 15860457]
39. Kuroda T, Tada M, Kubota H, et al. Octamer and Sox elements are required for transcriptional cis regulation of Nanog gene expression. *Mol Cell Biol.* 2005; 25:2475–2485. [PubMed: 15743839]
40. Hawkins K, Mohamet L, Ritson S, Merry CL, Ward CM. E-cadherin and, in its absence, N-cadherin promotes Nanog expression in mouse embryonic stem cells via STAT3 phosphorylation. *Stem Cells.* 2012; 30:1842–1851. [PubMed: 22696497]
41. Zhou JJ, Chen RF, Deng XG, et al. Hepatitis C virus core protein regulates NANOG expression via the stat3 pathway. *FEBS Lett.* 2014; 588:566–573. [PubMed: 24462277]

42. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature*. 2000; 408:307–310. [PubMed: 11099028]
43. Oren M. Decision making by p53: life, death and cancer. *Cell Death Differ*. 2003; 10:431–442. [PubMed: 12719720]
44. Goh AM, Coffill CR, Lane DP. The role of mutant p53 in human cancer. *J Pathol*. 2011; 223:116–126. [PubMed: 21125670]
45. Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol*. 2007; 17:165–172. [PubMed: 17196391]
46. Stecca B, Ruiz i Altaba A. A GLI1-p53 inhibitory loop controls neural stem cell and tumour cell numbers. *EMBO J*. 2009; 28:663–676. [PubMed: 19214186]
47. Bagga S, Bracht J, Hunter S, et al. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. *Cell*. 2005; 122:553–563. [PubMed: 16122423]
48. Makeyev EV, Maniatis T. Multilevel regulation of gene expression by microRNAs. *Science*. 2008; 319:1789–1790. [PubMed: 18369137]
49. Sirotkin AV, Laukova M, Ovcharenko D, Brenaut P, Mlyncek M. Identification of microRNAs controlling human ovarian cell proliferation and apoptosis. *J Cell Physiol*. 2010; 223:49–56. [PubMed: 20039279]
50. Lee YS, Kim HK, Chung S, Kim KS, Dutta A. Depletion of human micro-RNA miR-125b reveals that it is critical for the proliferation of differentiated cells but not for the down-regulation of putative targets during differentiation. *J Biol Chem*. 2005; 280:16635–16641. [PubMed: 15722555]
51. Hatfield SD, Shcherbata HR, Fischer KA, Nakahara K, Carthew RW, Ruohola-Baker H. Stem cell division is regulated by the microRNA pathway. *Nature*. 2005; 435:974–978. [PubMed: 15944714]
52. Liu C, Kelnar K, Liu B, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med*. 2011; 17:211–215. [PubMed: 21240262]
53. Tay YM, Tam WL, Ang YS, et al. MicroRNA-134 modulates the differentiation of mouse embryonic stem cells, where it causes post-transcriptional attenuation of Nanog and LRH1. *Stem Cells*. 2008; 26:17–29. [PubMed: 17916804]
54. Bicker S, Khudayberdiev S, Weiss K, Zocher K, Baumeister S, Schrat G. The DEAH-box helicase DHX36 mediates dendritic localization of the neuronal precursor-microRNA-134. *Genes Dev*. 2013; 27:991–996. [PubMed: 23651854]
55. Zhong J, Li B. Reduced expression of microRNA-134 correlates with malignancy and poor prognosis in human glioma. *J Clin Neurosci*. 2015; 22:583–587. [PubMed: 25564273]
56. Niu CS, Yang Y, Cheng CD. MiR-134 regulates the proliferation and invasion of glioblastoma cells by reducing Nanog expression. *Int J Oncol*. 2013; 42:1533–1540. [PubMed: 23467648]
57. Smirnova L, Grafe A, Seiler A, Schumacher S, Nitsch R, Wulczyn FG. Regulation of miRNA expression during neural cell specification. *Eur J Neurosci*. 2005; 21:1469–1477. [PubMed: 15845075]
58. Venkataraman S, Alimova I, Fan R, Harris P, Foreman N, Vibhakar R. MicroRNA 128a increases intracellular ROS level by targeting Bmi-1 and inhibits medulloblastoma cancer cell growth by promoting senescence. *PLoS One*. 2010; 5:e10748. [PubMed: 20574517]
59. Ciafre SA, Galardi S, Mangiola A, et al. Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem Biophys Res Commun*. 2005; 334:1351–1358. [PubMed: 16039986]
60. Masri S, Liu Z, Phung S, Wang E, Yuan YC, Chen S. The role of microRNA-128a in regulating TGFbeta signaling in letrozole-resistant breast cancer cells. *Breast Cancer Res Treat*. 2010; 124:89–99. [PubMed: 20054641]
61. Khan AP, Poisson LM, Bhat VB, et al. Quantitative proteomic profiling of prostate cancer reveals a role for miR-128 in prostate cancer. *Mol Cell Proteomics*. 2010; 9:298–312. [PubMed: 19955085]
62. Woo HH, Laszlo CF, Greco S, Chambers SK. Regulation of colony stimulating factor-1 expression and ovarian cancer cell behavior in vitro by miR-128 and miR-152. *Mol Cancer*. 2012; 11:58. [PubMed: 22909061]

63. Jin M, Zhang T, Liu C, et al. miRNA-128 suppresses prostate cancer by inhibiting BMI-1 to inhibit tumor-initiating cells. *Cancer Res.* 2014; 74:4183–4195. [PubMed: 24903149]
64. Fujii T, Shimada K, Tatsumi Y, Fujimoto K, Konishi N. Syndecan-1 responsive microRNA-126 and 149 regulate cell proliferation in prostate cancer. *Biochem Biophys Res Commun.* 2015; 456:183–189. [PubMed: 25462564]
65. Xu CX, Xu M, Tan L, et al. MicroRNA miR-214 regulates ovarian cancer cell stemness by targeting p53/Nanog. *J Biol Chem.* 2012; 287:34970–34978. [PubMed: 22927443]
66. Penna E, Orso F, Cimino D, et al. microRNA-214 contributes to melanoma tumour progression through suppression of TFAP2C. *EMBO J.* 2011; 30:1990–2007. [PubMed: 21468029]
67. Yang H, Kong W, He L, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. *Cancer Res.* 2008; 68:425–433. [PubMed: 18199536]
68. Mathieu J, Zhang Z, Zhou W, et al. HIF induces human embryonic stem cell markers in cancer cells. *Cancer Res.* 2011; 71:4640–4652. [PubMed: 21712410]
69. Wu CP, Du HD, Gong HL, et al. Hypoxia promotes stem-like properties of laryngeal cancer cell lines by increasing the CD133+ stem cell fraction. *Int J Oncol.* 2014; 44:1652–1660. [PubMed: 24573690]
70. Hasmim M, Noman MZ, Lauriol J, et al. Hypoxia-dependent inhibition of tumor cell susceptibility to CTL-mediated lysis involves NANOG induction in target cells. *J Immunol.* 2011; 187:4031–4039. [PubMed: 21911602]
71. Moretto-Zita M, Jin H, Shen Z, Zhao T, Briggs SP, Xu Y. Phosphorylation stabilizes Nanog by promoting its interaction with Pin1. *Proc Natl Acad Sci USA.* 2010; 107:13312–13317. [PubMed: 20622153]
72. Brumbaugh J, Russell JD, Yu P, Westphall MS, Coon JJ, Thomson JA. NANOG is multiply phosphorylated and directly modified by ERK2 and CDK1 in vitro. *Stem Cell Reports.* 2014; 2:18–25. [PubMed: 24678451]
73. Kim SH, Kim MO, Cho YY, et al. ERK1 phosphorylates Nanog to regulate protein stability and stem cell self-renewal. *Stem Cell Res.* 2014; 13:1–11. [PubMed: 24793005]
74. Xie X, Piao L, Cavey GS, et al. Phosphorylation of Nanog is essential to regulate Bmi1 and promote tumorigenesis. *Oncogene.* 2014; 33:2040–2052. [PubMed: 23708658]
75. Chu WK, Dai PM, Li HL, Pao CC, Chen JK. Nanog expression is negatively regulated by protein kinase C activities in human cancer cell lines. *Carcinogenesis.* 2013; 34:1497–1509. [PubMed: 23536578]
76. Ho B, Olson G, Figel S, Gelman I, Cance WG, Golubovskaya VM. Nanog increases focal adhesion kinase (FAK) promoter activity and expression and directly binds to FAK protein to be phosphorylated. *J Biol Chem.* 2012; 287:18656–18673. [PubMed: 22493428]
77. Ramakrishna S, Suresh B, Lim KH, et al. PEST motif sequence regulating human NANOG for proteasomal degradation. *Stem Cells Dev.* 2011; 20:1511–1519. [PubMed: 21299413]
78. Wu Y, Guo Z, Wu H, et al. SUMOylation represses Nanog expression via modulating transcription factors Oct4 and Sox2. *PLoS One.* 2012; 7(6):e39606. [PubMed: 22745796]
79. Paranjape AN, Balaji SA, Mandal T, et al. Bmi1 regulates self-renewal and epithelial to mesenchymal transition in breast cancer cells through Nanog. *BMC Cancer.* 2014; 14:785. [PubMed: 25348805]
80. Kregel S, Szmulewitz RZ, Griend DJ. The pluripotency factor Nanog is directly upregulated by the androgen receptor in prostate cancer cells. *The Prostate.* 2014; 74:1530–1543. [PubMed: 25175748]

Figure 1. Regulation of NANOG expression in cancer cells

Presented are several reported scenarios whether NANOG expression is regulated at the transcriptional, mRNA, and protein levels.

At the transcriptional level, AR (androgen receptor) binds the promoter of *Nanog* and enhances its expression. Hypoxia increases the expression of Nanog through HIF binding the promoter of Nanog. HCV core increases the activity of STAT3 and improves the binding of pSTAT3 to the regulatory region of Nanog to induce its expression. PKCα and PKCδ suppress the binding of OCT4-SOX composite to the promoter of *Nanog*, and therefore

exhibit Nanog transcription. In mouse ES cells, p53 directly binds to the Nanog gene promoter to repress its expression. In human GBM stem cells, NANOG and GLI transcription factors form a positive feedback loop that positively regulate the transcription of each other and is suppressed by p53.

At the mRNA level, most miRNAs (miR-126, -128, -134, -149) directly bind to the complementary sites in the 3'-untranslated region (3'-UTR) of the Nanog gene, and induce the translational repression or transcript degradation. However, miR-214 enhances the Nanog expression through mediating the degradation of p53. This mechanism of regulation may be applicable to both NANOG1 and NANOGP8.

At the protein level, PKC ϵ enhances the activity of NANOG by phosphorylating NANOG protein at Thr 200 and Thr 280. NANOG also forms complex with FAK, which, in turn, phosphorylates NANOG at Tyr 35 and Tyr 174, and hence improves its activity. ND, N-terminal domain; HD, homeodomain; CD1, C1-terminal domain; WR, tryptophan-rich domain; CD2, C2-terminal domain.

Figure 2. Examples of NANOGP8 regulation in cancer cells

This figure presents the so far reported examples of how NANOGP8 could be potentially regulated in cancer cells. In prostate cancer cells, hypoxia increases NANOGP8 mRNA through HIF. On the other hand, in GBM cells, NANOGP8 forms a positive feedback loop with Gli1, which is antagonized by p53. See Text for details.