Dissecting the transcriptional regulatory networks of promoter-associated noncoding RNAs in development and cancer

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Abstract

In-depth analysis of global RNA sequencing has enabled a comprehensive overview of cellular transcriptomes and revealed the pervasive transcription of divergent RNAs from promoter regions across eukaryotic genomes. These studies disclosed that genomes encode a vast repertoire of RNAs beyond the well-known protein-coding messenger RNAs. Furthermore, they have provided novel insights into the regulation of eukaryotic epigenomes, and transcriptomes, including the identification of novel classes of noncoding transcripts, such as the promoter-associated noncoding RNAs (pancRNAs).

PancRNAs are defined as transcripts transcribed within few hundred bases from the transcription start sites (TSSs) of protein-coding or non-coding genes. Unlike the long trans-acting ncRNAs that regulate expression of target genes located in different chromosomal domains and displaying their function both in the nucleus and in the cytoplasm, the pancRNAs operate as cis-acting elements in the transcriptional regulation of neighboring genes. PancRNAs are very recently emerging as key players in the epigenetic regulation of gene expression programs in development and diseases.

Herein, we review the complex epigenetic network driven by pancRNAs in eukaryotic cells, their impact on physiological and pathological states, which render them promising targets for novel therapeutic strategies.

Keywords: Promoter-associated noncoding RNA (pancRNA), Chromatin remodeling, Epigenome, Development, Cancer

Background

Transcription initiation is a tightly regulated process that involves remodeling of nucleosome organization and recruitment of transcription factors and the RNA Polymerase II (RNAPII) on the transcription units. Recent advances based on RNA sequencing efforts documented several classes of long and short noncoding RNAs transcribed in the promoter regions proximal the transcription start sites (TSS) of most annotated genes, bona fide involved in the regulation of transcription initiation [1]. Among them, the promoter-associated noncoding RNAs (named interchangeably pancRNAs or pncRNAs) have now been described in all eukaryotic species, from yeast to human [1–9]. PancRNAs are defined as transcripts transcribed within a few hundred bases from the TSSs of protein-coding or non-coding genes. Unlike the long trans-acting ncRNAs that regulate expression of target genes located in different chromosomal domains or even...
in different chromosomes, the pancRNAs by definition operate as cis-acting elements in the transcriptional regulation of neighboring genes [10]. Notably, these noncoding transcripts often show tissue specificity, suggesting regulated expression and functional involvement in biological processes [10].

Here we are reviewing current knowledge about cis-acting ncRNAs transcribed from promoter regions of protein-coding genes and their impact on chromatin signature and gene expression programs, in both physiological and pathological conditions.

Mechanisms of transcriptional regulation by pancRNAs

The first evidence documenting the presence of noncoding transcripts arising from regions located upstream the TSS came from high throughput experiments where hRrp40, a core component of the human 3' to 5' exoribonucleolytic exosome and one of the major RNA degradation complexes, was depleted [11]. In these studies, orientation-specific RT-qPCR performed on RNA from either hRrp40 depleted- or control cells demonstrated that both sense and antisense transcripts were detectable upstream the TSS, and the transcribed region was characterized by markers of active transcription, such as RNA polymerase II (RNAPII) and acetylated histone 3 (H3K9ac) peaks [11]. Genome-wide and global nuclear run on sequencing confirmed the presence of a population of low-copy small RNAs transcribed from promoter regions of the mammalian genome, shorter than 200 nucleotides, and produced by non-overlapping bidirectional transcription sites [2, 3, 12]. Promoter regions are intrinsically bidirectional [13]. Bidirectionality is achieved through the generation of nucleosome depleted regions via recruitment of nucleosome remodeling complexes that stimulate transcription in both directions [14]. However, sense and antisense divergent transcription rates do not correlate [13] and directionality of transcription units is controlled by a number of regulators, including the Chromatin Assembly Factor 1 (CAF-1) and factors that promote H3K56 acetylation [15], whereas antisense transcription positively correlates with H4 acetylation [13]. A fascinating hypothesis is that these bidirectional transcripts may act as a RNA reservoir forged by evolutionary pressures to support physiological functions [16, 17].

How these noncoding transcripts, both sense and antisense, are involved in the regulation of their host genes is still under investigation, and multiple mechanisms have been proposed, leading either to the activation or repression of the host genes. One hypothesis is that pancRNAs act as mediators of sequence-specific epigenetic changes. To this regard, one proposed molecular mechanism involves the control of CpG (de) methylation, as described for the Sphk1 gene and its Khps1 pancRNA variants [18]. A similar mechanism was observed in mouse embryos, where expression of bidirectional pancRNAs was strongly associated with the upregulation of their host genes during the zygotic genome activation at the 2-cell stage accompanied by sustained DNA demethylation [19], potentially driven by trans-acting factors, such as TET (Ten Eleven-Translocation, Methylcytosine Dioxygenase) proteins, to achieve a sequence-specific DNA demethylation (Fig. 1a).

An alternative, but not mutually exclusive possibility, is that pancRNAs display a more general function, facilitating rapid activation or repression of the downstream gene by altering chromatin structure or by recruiting transcription regulators. Short RNAs originating within 700 base pairs (bp) upstream the TSS of genes targeted by Polycomb were identified in primary T cells and embryonic stem cells [20]. These RNAs form a stem-loop structure interacting with SUZ12, a component of the Polycomb repressive complex 2 (PRC2). Recruitment of the complex achieves histone H3 Lys27 trimethylation (H3K27me3) thus causing repression of the gene [20, 21] (Fig. 1b).

Another functional model derives from initial work performed in S. pombe [22] and confirmed in humans [23], where the pancRNAs variants are involved in RNA-mediated contribution to the recruitment of transcription factors to enhancers and promoters. This model would suggest that bidirectional transcription of active enhancers and promoters evolved to facilitate trapping of transcription factors at specific regulatory elements, producing a positive feedback loop that contribute to the establishment of gene expression programs (Fig. 1c).

In a further model, the RNAPII reading through the promoter allows transcription of low copy pancRNAs targeted by antisense RNAs. The pancRNAs and antisense RNA form a complex that associate with the local chromatin architecture through a chromatin remodeling complex presumably containing histone methyltransferase DNMT3A. Remarkably, the antisense RNAs-pancRNAs form RNA:RNA hybrids that create docking sites for the recruitment of gene silencing complexes [24, 25]. In particular, in this model pancRNAs containing an extended 5’ UTR are recognized by endogenous antisense RNAs during RNAPII-mediated transcription of the RNA-targeted promoter. The antisense strand guides a silencing complex composed by DNMT3A, Ago-1, HDAC-1, and/or EZH2 to the targeted promoter (Fig. 1d). Next, the antisense RNA-targeted promoter may result in heterochromatinization of the local siRNA-targeted genomic region, exhibiting a silent shape, marked by both histone H3 lysine-9 dimethylation (H3K9me2) and histone H3 lysine-27 trimethylation (H3K27me3) [24].
In addition to RNAPII, pancRNAs transcribed by RNAPI in the nucleolus have been shown to take part to regulatory networks controlling the epigenetic state of chromatin of ribosomal genes. These pancRNAs form a DNA-RNA triplex recognized by the DNA methyltransferase DNMT3B to achieve de novo CpG methylation of rRNA genes (rDNA) [26]. The formation of DNA-RNA hybrid structures has been proposed to explain the unmethylated status of most human CpG islands in promoter regions. Unlike the previous model, unmethylated CpG promoters in the human genome show strand asymmetry in the distribution of guanine and cytosines, a property known as “GC skew”. This property confers the ability to form stable, three-stranded structures called R-loops upon RNA transcription. R-loop formation at promoters can prevent binding of DNMT3B and, therefore, DNA methylation [27].

Another mechanism proposed for pancRNA function has been illustrated for the cyclin D1 gene (CCND1) [28, 29]. Wang and colleagues described DNA damage-driven pancRNAs transcribed in HeLa cells from the region upstream the TSS of CCND1 gene. The authors showed that the pancCCND1_D was able to recruit the RNA binding protein FUS and CBP/p300, thus inhibiting the histone acetyltransferase activity of the p300/CREB binding protein (CBP)-associated factor (PCAF) and resulting in the suppression of transcription.

pancRNAs: regulators of transcription in developmental processes

In the past few years, pancRNAs have been shown to cover crucial roles in the guidance of cell differentiation [18, 19, 32, 33]. Transcriptome profiling of human and murine embryonic stem cells revealed that more than 60% of the lncRNA derive from divergent transcription at the promoters of protein-coding genes. Analysis of the distribution of these divergent pancRNAs revealed that their position in the genome is not casual, but it is correlated with protein-coding genes enriched in functions linked to developmental processes [34]. In fact, the pancRNA/mRNA gene pairs undergo coordinated transcriptional changes during the differentiation of the human embryonic stem cells toward endoderm [23]. Furthermore, transcriptome signatures revealed that bidirectional pancRNAs display different expressions pattern in human fetal and adult heart, due to a fine-tuned orchestration of specific epigenetic modifications, suggesting that pancRNAs which show a mutual expression with their neighboring genes, may contribute to the fetal heart development [35].

It is well known that promoters with a high CpG content can undergo bidirectional transcription and that divergent lncRNAs are capable to positively regulate in cis the transcription of the nearby coding genes, by contributing to their epigenetic setting [33]. As mentioned above, one of the first evidence documenting the modulation of gene expression depending on the bidirectional activity and the methylation status of the promoter was provided for the sphingosine kinase 1 (Sphk1) gene, in primary cells from rat fetuses [18]. Sphingosine kinase 1 is involved in the differentiation of fibroblast and keratinocytes, as well as in vascular and neural development. The Sphk1 CpG island contains a regulatory element, indicated as tissue-dependent differentially methylated region (T-DMR), involved in the regulation of Sphk1 expression. Transcription of the antisense pancRNA Kyps1 from the promoter induces DNA demethylation at CG sites, specifically in the T-DMR of Sphk1, thus inducing de-repression of the gene [18]. A further proof that pancRNAs can induce the demethylation of CpG islands upstream the partnered ORF, came later from a study conducted in normal rat kidney epithelial cells and undifferentiated PC12 rat cell line [32]. This function of
the pancRNAs has been demonstrated as essential for the zygotic gene activation and the acquisition of pluripotency in mouse early embryos but also for meso- and endodermal differentiation of pluripotent murine embryonic stem cells [19, 36]. Initially, in mouse preimplantation embryos, the pancRNA  pancII17d turned out to be responsible for triggering the expression of the interleukin 17d, indispensable for successful formation of the blastocyst. Although the expression of this pancRNA was associated with an increased DNA demethylation at the promoter, the molecular mechanism underlying this regulation was not further characterized. The epigenetic modifications associated to the zygotic gene activation was assumed to be due to the enrollment of PARP and TET3 demethylation factors on chromatin by pancRNAs or to be possibly only an indirect upshot [19, 37].

In addition, it has been proposed that pancRNAs might bind epigenetic factors, promoting the fold of the chromatin into a transcriptionally competent ternary structure, named R-Loop and composed by the nascent RNA annealed with its complementary ssDNA and the non-template ssDNA. Another possibility was that pancRNAs may perhaps stabilize the active state of chromatin through the association with small dsRNAs, which in turn, could cover the catalytic site of methyl transferases [38].

A subsequent investigation carried out in murine ESC, underlined the requirement of Evx1as lncRNA for the transcriptional activation of the neighboring protein-coding gene EVX1, essential for mesoderm differentiation [36]. In this case, the authors successfully demonstrated that the pancRNA binds an enhancer site on its locus and enables the recruitment of the transcription co-activator Mediator on the promoter [36]. Mediator thus coordinates the enhancer–promoter gene loop required for the assembly of the transcription machinery (Fig. 1c) [36].

Coupled expression of pancRNAs and the relative mRNAs in differentiating cells is often supported by the enrichment of histone acetylation and methylation in the promoter [34]. The terminal differentiation of PC12 cells is cAMP-dependent and relies on the fine-tuned expression of several pancRNAs deriving from bidirectional promoters enriched in cAMP response elements [39]. Among them,  pancNusap1 positively regulates the expression of the spindle-associate gene  Nusap1, and this effect is accompanied by an increased acetylation of H3 lysine 9 and 27 at the promoter [39]. The stimulation with cAMP induces a CRE-mediated concerted repression of  pancNusap1 and  Nusap1, resulting in the inhibition of the M-phase and the irreversible cell cycle withdrawal [39]. Similarly,  Uph1 pancRNA regulates in cis the transcription of its paired coding gene  Hand2 by enhancing the histone modifications H3K4me1 and H3K27ac in the enhancer [40]. The function of  Uph1 is critical for heart development in mice embryos, as demonstrated by the severe cardiac defects of  Uph−/− mice, leading to embryonic lethality at stage E10.5 [40]. A possible mechanism explaining the increased histone acetylation comes from recent data documenting that during myogenic differentiation, the pancRNA  Myoparr binds the myogenin shared promoter and physically interacts with the transcriptional co-activator DDX17 and the histone acetylase PCAF. This association leads to increased H3K27 acetylation of the promoter and the subsequent recruitment of the transcription factor MYOD1 [41]. This also allows the maximum occupancy of the RNAPII on the Myogenin locus, contributing to the exit of myoblasts from cell cycle and promoting differentiation [41].

Remarkably, recruitment of the Mediator complex on the  EVX1 promoter is associated to the H3K27ac and H3K4me1 histone modifications [36], where the histone acetylases recruited by the antisense pancRNA empower the access of Mediator. Some antisense poly(A) + pancRNAs from bidirectional promoters can also antagonize the transcription of their relative sense mRNAs. As an example, the pancRNA  hTAPAS represses the gene encoding human telomerase reverse transcriptase (hTERT). In the germline and human embryonic stem cell line  hTAPAS is fundamental for keeping low the concentration of the TERT protein, guarantying telomere length homeostasis and proliferative potential in the cells [42]. It is interesting to note that some antisense pancRNAs do not regulate the expression of the nearby locus, but can modulate in trans the transcription of other genes, by functionally interacting with the protein encoded from their cognate sense transcript, as for the pancRNA  Six3OS, that modulates the activity of the transcriptional factor SIX3, by linking chromatin-modifying enzyme complexes on the SIX3 target genes [43]. This mechanism seems required for cell specification in the postnatal retina and the correct eye development in mice [43]. Interestingly, in the crustacean  Daphnia magna it has been recently characterized the sense capped but non-polyadenylated pancRNA  DAPALR, that can activate in trans the transcription of the downstream locus  dsx1, relevant for sex determination during embryogenesis [6]. The molecular mechanism underlying the action of  DAPALR is still unclear, but this discovery denotes that the developmental role of pancRNAs is conserved in all eukaryotes although displaying diversification for directional transcription, post-transcriptional modifications and mechanism of action. Interestingly, antisense lncRNAs and sense transcripts tend to have a concerted expression in a tissue-specific fashion in fully developed organs, including cerebral cortex, cerebellum, heart, kidney and liver [33, 34]. This trend is conserved between mammalian species (chimpanzee, macaque, marmoset, mouse and rat), suggesting that pancRNAs participate in
the maintenance of tissue homeostasis in adults [33, 34]. To this regard, it has been shown that pancRNAs undergo a strong activation and are involved in the response to oxidative stress in somatic cells [44]. In particular, after exposure to reactive oxygen species, pancRNAs associate with polyosomes in the cytoplasm and compete with mRNAs, lowering the translational efficiency and favoring stress adaptation [44].

All these evidences support the idea that pancRNAs display evolutionary conserved functions emblematic both for differentiation and homeostasis in all eukaryotes.

Cancer-related scaffolding function of pancRNAs

Alterations of pancRNAs have been extensively linked to the changes of gene expression programs occurring in human cancer. PancRNAs affect epigenetic modification of promoters through scaffolding function. It has been shown that non-protein coding transcripts display key roles in cancer biology by impacting the gene expression of important regulatory proteins [45]. In particular, emerging evidences report the ability of ncRNAs to fold into a tertiary structure providing a scaffold for other proteins or regulatory molecules, including RNAs [45].

It is well established the oncogene-like role of pancRNAs in tumorigenesis through the control of critical cell cycle regulators like cyclins [28, 29]. In response to DNA damage such as ionizing irradiation (IR), the CCND1 pancRNAs are transcribed and negatively regulate cyclin D1 expression [28]. In HeLa cells, pancRNAs transcribed from the promoter of CCND1 gene associate with and recruit the RNA binding protein FUS/TLS (Fused in sarcoma, Translocated in liposarcoma). The localization of the pancRNA_CCND1-FUS complex to the CCND1 promoter results in the inhibition of the CBP/p300 histone acetyltransferases activity thereby preventing CCND1 transcription [28]. More recently, a similar repression of the cyclin D1 expression mediated by a protein-RNA complex was described in Ewing sarcoma cells [29]. A deep investigation of the molecular mechanisms underlying this regulation showed that after mitogenic stimuli, such as IGF-1 treatment, the protein partners EWS-FLI1 and DHX9 interact promoting the transcription of target genes, such as CCND1. Perturbation of the EWS-FLI1/DHX9 interaction by the small molecule YK-4-279 causes the formation of an alternative inhibitory complex composed by the RNA binding protein Sam68, DHX9, and the pancRNA_CCND1_B determining a repressive chromatin environment responsible of cyclin D1 transcriptional inhibition. The ability of pancRNAs_CCND1 to affect cell proliferation through transcriptional regulation of CCND1 reveals the contribution of these molecules in the control of cell cycle progression and in the tumorigenic process [29].

A similar mechanism has been documented for the pancRNA_Ets-1, displaying a role in the promotion of in vivo/in vitro growth and aggressiveness of gastric cancer [46]. PancRNA_Ets-1 facilitates the physical interaction between the RNA binding protein NONO and the transcriptional factor EGFR, resulting in transactivation of EGFR and increased cancer-related gene transcription [46]. Moreover, pancRNA_Ets-1 displays a crucial role in neuroblastoma tumorigenesis, by promoting neuroblastoma cell growth, invasion and metastasis. PancRNA_Ets-1 binds to the heterogeneous nuclear ribonucleoprotein K (hnRNPK) to facilitate its physical interaction with β-catenin, resulting in reduced β-catenin proteasome-dependent degradation [47]. As a consequence, the enhanced nuclear translocation of β-catenin affects its gene transcriptional program contributing to neuroblastoma progression. Notably, pancRNA_Ets-1 upregulation in clinical tissues and cell lines of both gastric cancer and neuroblastoma could be exploited as promising markers and potential therapeutic targets.

RNA-directed transcriptional gene regulation by pancRNAs in cancer

The pancRNA_FOXCUT is transcribed upstream of the Forkhead box C1 (FOXC1) gene and its expression is positively correlated with FOXC1 mRNA [48]. The transcription factor FOXC1 is a key regulator of several biological processes and its abnormal expression was found associated to poor survival in various malignant tumors and in the epithelial to mesenchymal transition (EMT) process [24, 49, 50]. Recent studies showed that the FOXCUT–FOXC1 regulatory network is associated with tumorigenesis and cancer progression in esophageal squamous cell carcinoma (ESCC) [50], oral squamous cell carcinoma (OSCC) [51] and nasopharyngeal carcinoma (NPC) [52]. FOXCUT expression was found enhanced in Basal-Like Breast Cancers (BLBCs) but not in other non-basal like breast cancer subtypes, suggesting that FOXCUT may function as specific biomarker in BLBCs [53]. Notably, in all these different tumor contexts, knockdown of FOXCUT reduced the levels of FOXC1 mRNA, concurring with the observed inhibition of migration, invasion and the metastatic potential of cells.

Similarly, the expression of the pancRNA_HIF2PUT (HIF2PUT) was found correlated with the expression of the hypoxia-inducible factor-2α (HIF-2α), in osteosarcoma patients [54, 55]. HIF-2α has been associated with stem-like properties in stem cells and in cancer stem cells of different types of tumor [46, 56, 57]. Moreover, HIF2PUT knockdown is able to induce enhanced proliferation, migration and self-renewal of MG63 cells,
suggesting that HIF2PUT may suppress the properties of cancer stem cells in osteosarcoma by regulating the expression of HIF-2α [54]. In colorectal cancer specimens, the expression of HIF2PUT positively correlates with HIF-2α expression and HIF2PUT knockdown impairs the stem cell like properties of colorectal cancer stem cells by regulating HIF-2α expression [58].

In different prostate cancer cell lines and tumors, Pisignano and colleagues showed that the gene encoding E-cadherin (CDH1), known as an epithelial cell differentiation marker and a tumor suppressor, is epigenetically silenced contributing to the acquisition of key essential properties in the tumor development and progression. They found that a specific miRNA (isomiR-4534) guides the Argonaute1 (Ago1) binding to a precise site on the pancRNA_CDH1. In turn, Ago1 recruits the histone methyltransferase SUV39H1 to repress the promoter activity with the consequent transcriptional suppression. Importantly, a cancer-associated single nucleotide polymorphism (SNP, rs16260) facilitates the accessibility of the miRNA-Ago1 complex to the pancRNA_CDH1, increasing CDH1 gene silencing [59].

As mentioned above, pancRNAs can also be involved in the formation of a RNA:DNA hybrid (named R-loop) to affect gene expression. This kind of mechanism was found in colon cancer cells and in two different breast cancer cell lines, MCF7 and MCF10, where the pancRNA_VIM, an antisense noncoding transcript on Vimentin RNA (deposited as VIM-ASI), supports an open chromatin state by participating to the formation of R-loop. This structure facilitates the accessibility of Vimentin gene to the transcriptional machinery and enhances the NF-κB binding to Vimentin promoter, thus resulting in increased gene transcription [60].

Beside the possibility to form a R-loop, pancRNAs may be involved in the formation of a DNA-RNA triplex. Recently, it has been reported that the expression of the proto-oncogene SPHK1, involved in the control of apoptosis, requires a pancRNA, termed Khps1, transcribed in antisense orientation to SPHK1. Khps1 is bound to a homopurine stretch (TFR2), forming a DNA-RNA duplex able to interact with p300/CBP, thus establishing an open chromatin structure that facilitates the E2F1-mediated SPHK1 transcription [61].

Collectively, these studies (listed in Table 1) suggest a role of pancRNAs in carcinogenesis. The discovery of the precise mechanisms through which these molecules may modulate transcription of cancer-related genes could provide a deeper understanding of the complex integrated network that supports the tumor phenotype.

### Conclusions

Genome-wide transcriptomic analyses have enabled huge advances in the understanding of the intricate
transcriptional regulatory network driven by cis-acting noncoding transcripts on their target genes, highlighting enhancing and repressive activities exerted by the pancRNAs on target genes [62]. Accumulating evidence shows that changes in the repertoire of pancRNAs finely shape tissue-specific gene expression patterns [34]. Indeed, comparative transcriptome analyses revealed higher diversity of pancRNA- over mRNA-expression profiles, favoring an evolutionary diversification of the transcriptome according to a given species [34]. In parallel, promoter regions of pancRNA-hosting genes exhibit a higher level of sequence conservation than those of pancRNA-lacking genes [34]. Thus, pancRNAs could represent a new layer of species-dependent gene regulation mechanism that allows and contributes to the dynamic evolutionary process and adaptation of a species.

The differential expression of pancRNAs between tumor and benign specimens suggest their potential usage as diagnostic, prognostic or predictive biomarkers. A fascinating question is whether single-nucleotide polymorphisms (SNPs) in the promoter regions affect the function of these promoter-proximal transcripts, by impacting chromatin dynamics and transcription factor recruitment. To this regard, several disease-associated SNPs map to noncoding regions, including promoters [63, 64], but whether pancRNAs are involved in the disease process and the mechanistic link between noncoding SNPs and transcriptional regulation has not been clarified yet, although possible effects of deleting regulatory elements contained within the mutated regions could not be excluded. Furthermore, given the relevance of pancRNAs in developmental processes and in pathological conditions, the potential of the endogenous pancRNA as a therapeutic target could be further evaluated and exploited. For example, forced expression of pancRNAs could be instrumental for artificially manipulating epigenetic modification on specific candidate genes, to accomplish a targeted and functional disruption of aberrant methylation activity, with important implications in transcriptional reprogramming. Conversely, targeted deregulation of pancRNA-based transcriptional network could contribute to epigenetic silencing of tumor suppressor genes and disease progression, thus altering the epigenetic landscape of patients.

Collectively, the reported findings highlight pancRNAs as potential candidates for transcriptional reprogramming. To this regard, the epigenome profile could be rewritten by a set of epigenetic modifiers, including pancRNAs. Manipulations of these molecules could be employed in medical purposes.
transcript, functions as an inhibitor of osteosarcoma stem cells in vitro. Mol Med Rep. 2015;11(4):2534–40.
55. Zhao D, Wang S, Chu X, Han D. LncRNA HIF2PUT inhibited osteosarcoma stem cells proliferation, migration and invasion by regulating HIF2 expression. Artif Cells Nanomed Biotechnol. 2019;47(1):1342–8.
56. Yang J, Zhang X, Zhang Y, Zhu D, Zhang L, Li Y, Zhu Y, Li D, Zhou J. HIF-2α promotes epithelial-mesenchymal transition through regulating Twist2 binding to the promoter of E-cadherin in pancreatic cancer. J Exp Clin Cancer Res. 2016;35:26.
57. Yan L, Xu F, Dai CL. Relationship between epithelial-to-mesenchymal transition and the inflammatory microenvironment of hepatocellular carcinoma. J Exp Clin Cancer Res. 2018;37(1):203.
58. Yao J, Li J, Geng P, Li Y, Chen H, Zhu Y. Knockdown of a HIF-2α promoter upstream long noncoding RNA impairs colorectal cancer stem cell properties in vitro through HIF-2α downregulation. Onco Targets Ther. 2015;8:3467–74.
59. Pisignano G, Napoli S, Magistri M, Mapelli SN, Pastori C, Di Marco S, Civenni G, Albino D, Enriquez C, Allegrini S, Mitra A, D’Ambrosio G, Mello-Grand M, Chiorino G, Garcia-Escudero R, Farani G, Carbone GM, Catapano CV. A promoter-proximal transcript targeted by genetic polymorphism controls E-cadherin silencing in human cancers. Nat Commun. 2017;8:15622.
60. Boque-Sastre R, Soler M, Oliveira-Mateos C, Portela A, Mourinho C, Sayols S, Villanueva A, Esteller M, Guil S. Head-to-head antisense transcription and R-loop formation promotes transcriptional activation. Proc Natl Acad Sci U S A. 2015;112(18):5785–90.
61. Postepska-Igielska A, Giwojna A, Gasri-Plotnitsky L, Schmitt N, Dold A, Ginsberg D, Grummt I. LncRNA Khps1 regulates expression of the proto- oncogene SPHK1 via triplex-mediated changes in chromatin structure. Mol Cell. 2015;60(4):626–36.
62. Bogu GK, Vizán P, Stanton LW, Beato M, Di Croce L, Marti-Renom MA. Chromatin and RNA maps reveal regulatory long noncoding RNAs in mouse. Mol Cell Biol. 2015;36(5):809–19.
63. M.T. Maurano, R. Humbert, E. Rynes, R.E. Thurman, E. Haugen, H. Wang, A.P. Reynolds, R. Sandstrom, H. Qu, J. Brody, A. Shafer, F. Neri, K. Lee, T. Kutyavin, S. Stehling-Sun, A.K. Johnson, T.K. Canfield, E. Giste, M. Diegel, D. Bates, R.S. Hansen, S. Neub, P.J. Sabo, S. Heimfeld, A. Pauzschek, S. Ziegler, C. Coutapak, N. Sotoodehnia, T. Glass, S.R. Sunyaev, K. Kall, J.A. Stamatoyannopoulos, Systematic localization of common disease-associated variation in regulatory DNA, Science 337(6099) (2012) 1190–5.
64. Nguyen Q, Carninci P. Expression specificity of disease-associated lncRNAs toward personalized medicine. Curr Top Microbiol Immunol. 2016;394:237–58.

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