

Gene Section

Review

NANOG (Nanog homeobox)

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Identity

HGNC (Hugo): NANOG

Location: 12p13.31

DNA/RNA

Description

The NANOG gene encompasses 8265 bp of DNA in humans and is located on the short arm of chromosome 12 (12p13.31) from 7940390 - 7948655. A 299 bp large NANOG promoter region (-264 to +35) upstream of exon 1 (Fig. 1A) contains five CpG-dinucleotides, which are subjected to DNA-methylation (Nettersheim et al., 2011a). Furthermore, this NANOG promoter region contains an OCT3/4-SOX2 binding motif, a TATA-box and binding sites for the transcription factors AP-2, SP1 and TFIID (Rodda et al., 2005; Nettersheim et al., 2011a) (PromoterScan 1.7). Functionality of this NANOG promoter element has been demonstrated by Luciferase-reporter experiments (Nettersheim et al., 2011a). A similar region (-229 to +193) has also been analysed by Wu et al. in mice (Wu da and Yao, 2005). There, binding sites for AP-1, SP1 and Oct3/4 could be found and functionality of this region in regulation of Nanog expression was demonstrated in F9 EC cells by Luciferase-assays.

Transcription

The mRNA encoded by NANOG has four transcript variants (Fig. 1B). Transcript variants NANOG-001 (2101 bp mRNA) and -002 (870 bp mRNA) are known protein coding, while variant -004 (561 bp mRNA) is

putatively protein coding and variant -003 is known as nonsense mediated decay. NANOG transcription can be regulated by binding of OCT3/4 and SOX2 to their binding motifs in the NANOG promoter (see Fig. 1A) (Nettersheim et al., 2011a; Kuroda et al., 2005; Rodda et al., 2005).

Pseudogene

Ten NANOG-related nucleotide sequences (NANOGP2 - NANOGP11) plus one non-functional duplication (NANOGP1) have been discovered in the human genome (Booth and Holland, 2004). NANOGP2 - NANOGP11 are processed but are lacking introns, except NANOGP8 which is a retrogene.

Protein

Description

NANOG mRNA variants NANOG-001 and -002 encode for a protein with a length of 305 amino acid (aa) and 289 aa, respectively. NANOG-004 is putatively protein coding; translation would result in a 186 aa long protein.

NANOG-001, the 305 aa long protein with a molecular weight of 34.6 kDa is usually analyzed to study the role of NANOG (Fig. 2A). It consists of a Serine-, Threonine- and Proline-rich N-terminal region as well as eight W-repeats at its C-terminus (aa 104-151). The DNA-binding facilitating homeodomain spans from aa 95-155. Formation of secondary structures (helix, strand and turn) occurs mainly within the homeobox-coding region (Fig. 2B).

The NANOG protein is unstable with a half-life of 120 min in human ESCs (Ramakrishna et al., 2011).

A)

5'-GATTTAAAAGTTGGAAACG**TGGTGAACCTAG**AAGTATTGTTGCTGGGTTTGTCTTCAGG
 TTCTGTTGCTCGGTTTTCTAGTTCCCCACCTAGTCTGGGTTACTCTGCAGCTACTTTTGC
ATTACAATGGCCTTGGTGAGACTGGTAGACCGGGATTA**ACTGAGAATT**CACAAGGGTGGGT
 CAGTAGGGGGTGTGCCCGCCAGGAGGGGGTGGGICTAAGGTGATAGAGCCTTCAT**TATAAA**
 TCTAGAGACTCCAGGATTTTAACG**TCTGCTGGACTGAGCTGGT**GCCTCATGTTATTAT-3'

- CpG-Dinucleotide
 - SOX2 binding motif
 - Transcription start site
 - AP-2 binding motif
- OCT3/4 binding motif
 - TATA-box
 - SP1 binding motif
 - TFIID binding motif

B)

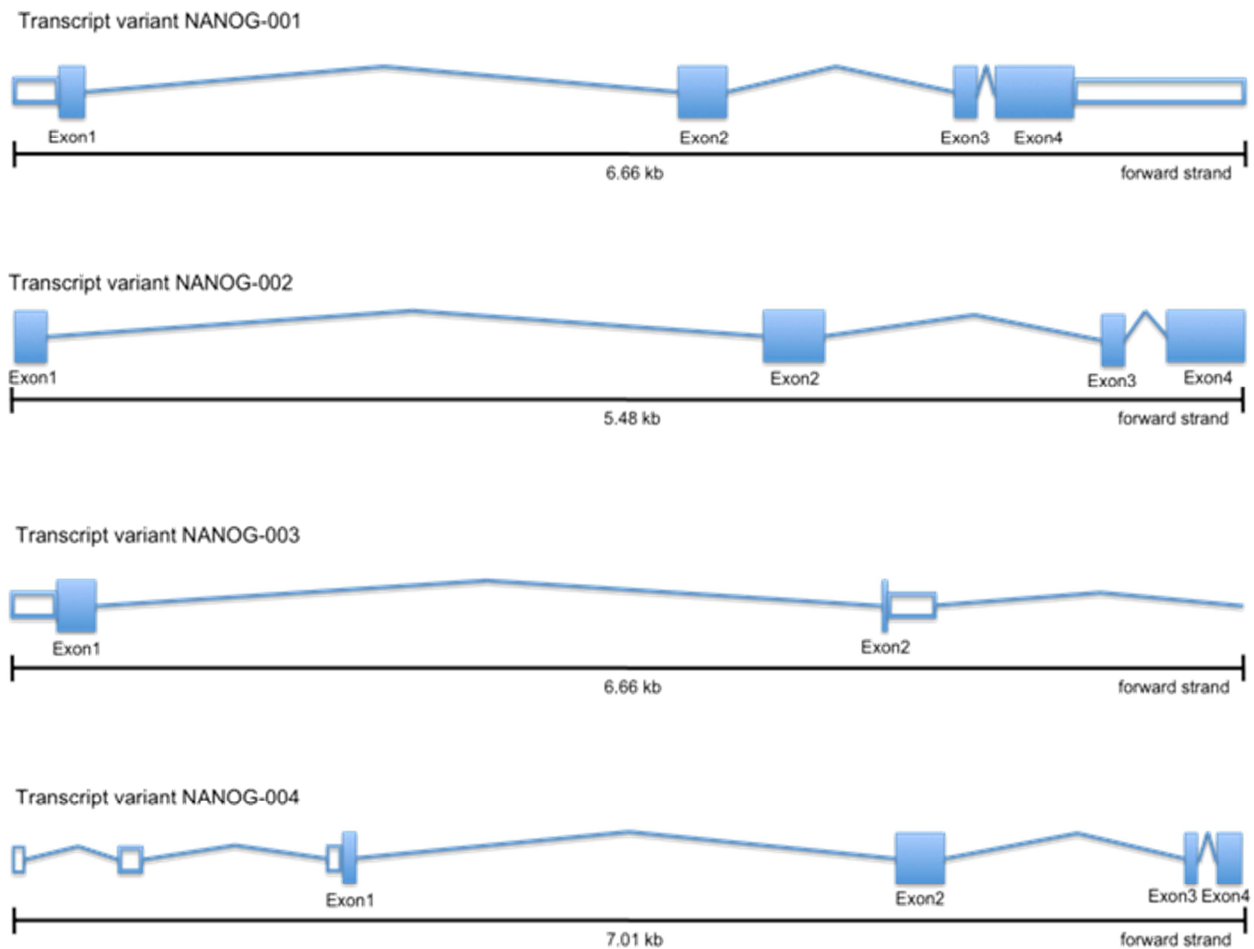


Figure 1. (A) Genomic sequence of the human NANOG promoter region. Important regulatory elements, binding sites and CpG-dinucleotides are indicated in different colors. **(B)** Graphical illustration of the four different human NANOG transcripts.

Expression

NANOG is expressed in undifferentiated embryonic stem cells (ESCs) (Ezeh et al., 2005), glioma stem cells (Zbinden et al., 2010), fetal gonocytes (Hart et al., 2005; Hoei-Hansen et al., 2005), carcinoma-in-situ (CIS) of the testis (Hart et al., 2005; Hoei-Hansen et al., 2005), seminoma (Ezeh et al., 2005; Hoei-Hansen et al., 2005), embryonal carcinoma (Hoei-Hansen et al., 2005) and mammary carcinoma / breast cancer (Ezeh et al., 2005). Ezeh et al. found NANOG expression in adult germ cells throughout germ cell differentiation (spermatogonia to spermatids) (Ezeh et al., 2005).

In contrast to the data of Ezeh et al., Hoei-Hansen et al. were unable to detect NANOG protein in adult testis tissue (Hoei-Hansen et al., 2005). NANOG is also expressed in the seminoma cell line TCam-2 and the embryonal carcinoma cell lines 2102EP, NCCIT, Tera-1, Tera-2, NT2/D1 and 833Ke (Nettersheim et al., 2011a; Nettersheim et al., 2011b; Eckert et al., 2008; You et al., 2009; Deb-Rinker et al., 2005; Freberg et al., 2007).

In germ cell tumors (GCTs), NANOG expression is regulated by binding of OCT3/4 and SOX2 (SOX17 putatively in seminomas) to the NANOG promoter (Nettersheim et al., 2011a).

Furthermore, in GCTs NANOG expression can be epigenetically suppressed by DNA-methylation of CpG-dinucleotides in the promoter (Nettersheim et al., 2011a).

NANOG expression correlates to the differentiation status of GCTs, i.e. the more differentiated the GCT, the lower the NANOG expression (Nettersheim et al., 2011a).

Expression of the NANOG pseudogene NANOGP8 has been detected in uterine cervix tumor tissue, breast tumor tissue, urinary bladder tissue and smooth muscle cells as well as in the cell lines OS732, HepG3 and MCF-7 (Zhang et al., 2006; Ambady et al., 2010).

Expression of pseudogenes NANOGP4 and NANOGP5 has been demonstrated in the cell lines OS732, HepG3 and MCF-7, too (Zhang et al., 2006).

Localisation

Nuclear.

Function

NANOG promotes self-renewal and maintains pluripotency in undifferentiated cells, like ESCs (Boyer et al., 2005; Wang et al., 2006). Thereby, NANOG acts in concert with the transcription factors SOX2, OCT3/4 (POU5F1) and FOXD3 (Pan and Thomson, 2007) (Wang et al., 2006). NANOG activates expression of SOX2 and OCT3/4 (Pan and Thomson, 2007).

A NANOG knock-down leads to differentiation of

ESCs into the extraembryonic endoderm and trophoblast lineages (Hyslop et al., 2005), demonstrating NANOG's important role in repression of differentiation.

However, siRNA/shRNA-mediated reduction of the NANOG protein in the seminoma cell line TCam-2 is tolerated without induction of differentiation (Nettersheim et al., 2011b).

It is assumed that the observed upregulation of OCT3/4 and SOX17 compensates for reduced NANOG levels in TCam-2 cells (Nettersheim et al., 2011b).

NANOG-shRNA transduced cancer cells exhibit decreased long-term clonal and clonogenic growth, reduced proliferation and altered differentiation (Jeter et al., 2009).

Overexpression of NANOG in ESC enables their feeder-cell-free propagation for multiple passages during which the cells remain pluripotent (Darr et al., 2006).

NANOG is able to modulate gliomasphere clonogenicity and CD133+ stem cell behaviour (Zbinden et al., 2010).

There, NANOG is regulated by the Hedgehog-GLI signalling pathway. GLI1 requires NANOG activity to form a positive loop, which is negatively controlled by p53 and vice versa (Zbinden et al., 2010).

Besides SOX2 and OCT3/4, protein interaction databases predict interaction of NANOG with SALL4, KLF4, ZFP42, ISYNA1, LIN28, FAM48A, SMAD1, GATA6, RIF1, TRIM28, SMARCA4 and SMARCA2 (by similarity) (String 9.0, UniProt, I2D).

Homology

NANOG and its pseudogenes (except NANOGP8) are present at their expected orthologous chromosomal positions in the chimpanzee genome when compared to the human genome (Fairbanks and Maughan, 2006). NANOGP8 is not found in the genome of the chimpanzee (Fairbanks and Maughan, 2006). The homeodomain of the human NANOG protein shares 87% identity to the murine NANOG homeodomain (Hart et al., 2004). A sequence comparison of murine, cow, chimp and human Nanog/NANOG promoters revealed that the CpG-dinucleotides are completely conserved in chimp and human (Nettersheim et al., 2011a).

Mutations

Somatic

NANOG is localized on the short arm of chromosome 12, which is often gained in type II germ cell tumors, mostly due to isochromosome formation (i(12p)) (Looijenga et al., 2003).

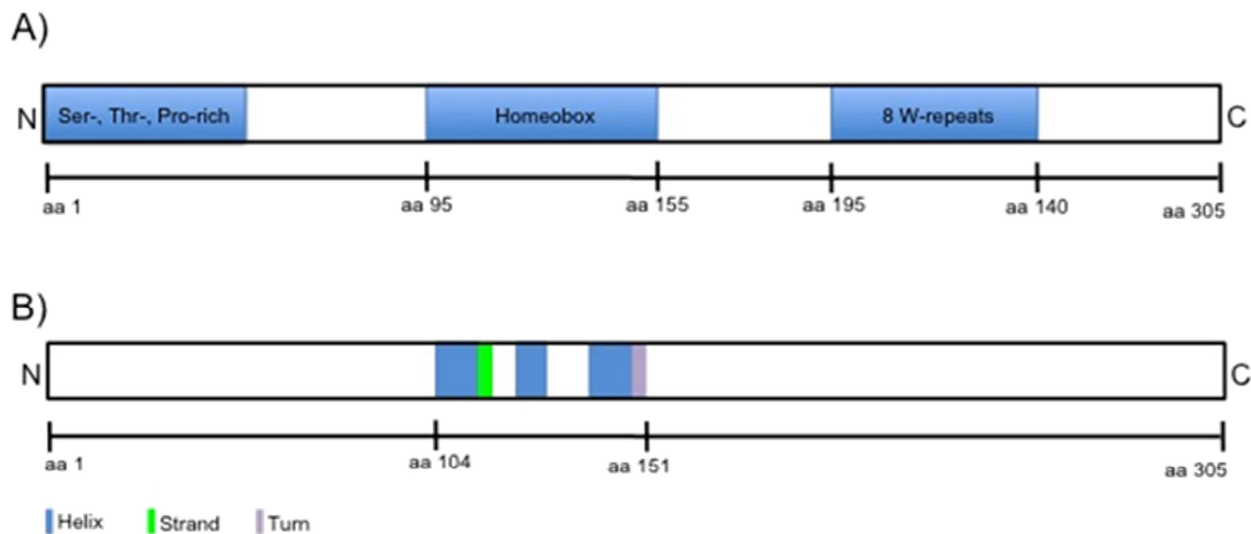


Figure 2: Graphical illustration of the NANOG protein sequence (A) and secondary structure motifs of the NANOG protein (B).

Implicated in

Testicular germ cell tumors

Oncogenesis

A consistent structural chromosomal abnormality in invasive testicular germ cell tumors are gains of the short arm of chromosome 12 (12p), due to isochromosome formation (Looijenga et al., 2003). This gain results in an increase in copy numbers of genes on 12p, which is speculated to lead to suppression of apoptosis and Sertoli-cell independency of CIS cells (Looijenga et al., 2003). NANOG is located in 12p and therefore amplified in invasive germ cell tumors. Increased NANOG copy numbers, resulting in overexpression of NANOG in seminomas and non-seminomas might lead to maintenance of self-renewal and mediates a pluripotency-like status. During differentiation of embryonal carcinomas into teratoma, choriocarcinoma and yolk-sac tumors the 12p gain is maintained, but NANOG expression is downregulated.

To be noted

Note

In the adult testis, global DNA-methylation levels decrease from spermatogonia (hypermethylated) to mature sperm (hypomethylated). Interestingly the NANOG promoter is hypomethylated in spermatogonia and hypermethylated in sperm (Nettersheim et al., 2011a). In sperm, it is hypothesized that NANOG promoter methylation represents a way to epigenetically repress NANOG expression in order to control the pluripotency program and to prevent germ cell malignancies (Nettersheim et al., 2011a).

NANOG promoter hypomethylation could be found in seminoma, embryonal carcinoma, fetal gonocytes as well as fetal and adult spermatogonia. NANOG promoter hypermethylation was demonstrated in adult

testis tissue, sperm, teratoma, choriocarcinoma, yolk-sac tumor and mixed non-seminoma (Nettersheim et al., 2011a). The cell lines TCam-2, 2102EP, Tera-1, Tera-2 and NT2/D1 harbor a hypomethylated NANOG promoter, while cell lines 833Ke, NCCIT and JKT-1 are hypermethylated at the NANOG promoter (Nettersheim et al., 2011a).

Upon differentiation of the seminoma cell line TCam-2 into a cell type resembling a mixed non-seminoma NANOG expression is downregulated (Nettersheim et al., 2011c).

NANOG has been shown to regulate self-renewal of cancer stem cells through the IGF pathway in hepatocellular carcinoma (Shan et al., 2012). Furthermore, increased expression of NANOG in cancer cells correlated to a worse clinical outcome. In addition, NANOG positive cancer stem cells were resistant to the therapeutic agents, sorafenib and cisplatin and have a high capacity for tumor invasion and metastasis.

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