Identifying SOX2-OT transcript that is responsible for regulating SOX2 in cancer cells and embryonic stem cells

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Abstract

SOX2 overlapping transcript (SOX2-OT) is an evolutionarily conserved long non-coding RNA (lncRNA) whose intronic region contains the transcript of pluripotency gene SRY-box transcription factor 2 (SOX2). It has been suggested that SOX2-OT can regulate its overlapping gene, SOX2. Studies demonstrated that elevated SOX2-OT promotes SOX2 expression in cancer cells, whereas levels of SOX2-OT are inversely correlated with levels of SOX2 in embryonic stem cells. It is not clear why there is a tremendous discrepancy in the regulation of SOX2 by SOX2-OT in cancer cells and embryonic stem cells. Due to the diversified transcription of the SOX2-OT gene, we hypothesize that differential expression of transcripts of the SOX2-OT gene in cancer cells and embryonic stem cells may contribute to the divergence in the regulatory relationship of SOX2-OT and SOX2. A CRISPR screening platform can be leveraged to systemic evaluate which transcript of the SOX2-OT gene may be responsible for upregulation or downregulation of SOX2 in cancer cells and embryonic stem cells, respectively.

Keywords

SOX2-OT, SOX2, Cancer cells, embryonic stem cells, CRISPR

Differential regulation of SOX2 by SOX2-OT in cancer cells and embryonic stem cells

SOX2 overlapping transcript (SOX2-OT) is an evolutionarily conserved long non-coding RNA (lncRNA) whose intronic region contains the transcript of pluripotency gene SRY-box transcription factor 2 (SOX2) (Amaral et al. 2009 ; Shahryari et al. 2015). It has been

indicated that lncRNA can regulate the expression of adjacent overlapping genes (Kopp and Mendell 2018 ; Marchese et al. 2017). Various studies have investigated the regulatory relationship between SOX2-OT and SOX2. Almost all cancer studies examining the function of SOX2-OT discovered that the elevation of SOX2-OT promotes SOX2 expression in cancer cells (Askarian-Amiri et al. 2014 ; Chang et al. 2020 ; Du et al. 2019 ; Hou et al. 2014 ; Hussenet et al. 2010 ; Li et al. 2018 ; Shahryari et al. 2014 ; Wang et al. 2020 ; Wang et al. 2017 ; Wei et al. 2018 ; Zhan et al. 2020 ; Zhang et al. 2017). However, studies regarding embryonic stem cells demonstrated that levels of SOX2-OT are inversely correlated with levels of SOX2 (Knauss et al. 2018 ; Messemaker et al. 2018). It is not clear why there is a tremendous discrepancy in the regulation of SOX2 by SOX2-OT in cancer cells and embryonic stem cells.

The human SOX2-OT gene is comprised of many exons and has multiple transcription start sites (TSSs) that exhibit complicated transcriptional features. Six RefSeq mRNAs with 15 additional mRNAs sequence are collected in Genebank (NCBI). According to Ensembl Genome Database, the human SOX2-OT gene expresses 104 mRNA-like transcripts, the longest of which is approximately 4.3 kb. In addition, RNAcentral (https://rnacentral.org/) and LNCipedia (https://lncipedia.org/) include 167 and 127 SOX2-OT transcripts, respectively. The diversified transcription increases the complexity of the SOX2-OT gene. We speculate that differential expression of transcripts of the SOX2-OT gene in cancer cells and embryonic stem cells may contribute to the divergence in the regulatory relationship of SOX2-OT and SOX2. Although some studies revealed the exact transcript of SOX2-OT can control SOX2 expression, those experiments generally studied how SOX2-OT regulates SOX2 expression by siRNA silencing (Chang et al. 2020, Knauss et al. 2018, Zhang et al. 2017). However, those studies did not consider the possibility that one siRNA may target two or three transcripts, which led to the imprecision.

A method to identify SOX2-OT transcript that is responsible for regulating SOX2 in cancer cells and embryonic stem cells

We can deploy a platform that leverages a CRISPR screening to systemic evaluate which transcript of the SOX2-OT gene may be responsible for upregulation or downregulation of SOX2 in cancer cells and embryonic stem cells, respectively. We need to avoid the use of CRSPR/Cas9 system because genomic editing to disturb the expression of SOX2-OT may affect the expression of SOX2. SOX2 is embedded in an intron of SOX2-OT and the regulatory elements to control SOX2 expression may localize in the gene body of SOX2-OT.

CRISPR/Cas13 system has been engineered to induce RNA knockdown without genomic modifications (Abudayyeh et al. 2017; Abudayyeh et al. 2016; East-Seletsky et al. 2016; Konermann et al. 2018; Smargon et al. 2017; Wessels et al. 2020). We can leverage CRISPR/Cas13 system to generate a screening platform to target the transcript of the SOX2-OT gene by designing unique CRISPR RNAs (crRNAs) targeting each

transcript. Due to every transcript has an exclusive combination of exons, crRNAs can be designed to target the adjacent region of two exons to obtain a particular crRNA targeting only one transcript. Designed crRNAs can be pooled to generate a lentivirus-based CRISPR library. Cancer cells or embryonic stem cells, which will be used for CRISPR screening, need to be engineered to express a GFP protein to easily track SOX2 levels by fluorescence-activated cell sorting (FACS). We can insert GFP cDNA into the end of the coding region of the SOX2 gene to induce a simultaneous co-expression of SOX2 and GFP. Engineered cancer cells or embryonic stem cells will be infected by the CRISPR library. After the selection of infected cells, a flow cytometer will be applied to isolate GFP highly expressed cells or lowly expressed cells compared to controls, in which cells will not be infected by the CRISPR library. A high GFP signal indicates upregulation of SOX2, whereas a low GFP signal indicates downregulation of SOX2. By analyzing crRNAs levels in GFP^{high} cells or GFP^{low} cells, we will know which transcript controls SOX2 expression in cancer cells or embryonic stem cells (Fig. 1). The results could be varified by co-transfection of Cas13 and identified crRNA in cancers cells and embryonic stem cells.

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Conflicts of interest

The authors declare no conflicts of interest.

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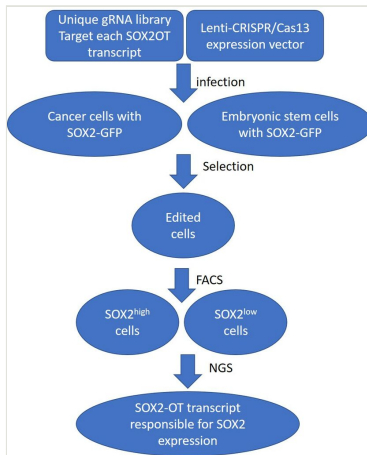
Figure 1.

The schematic diagram for CRISPR/Cas13-based screening system for identification of SOX2-OT transcript that differentially controls SOX2 expression in cancer cells and embryonic stem cells.