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Evidence for a role of a lncRNA encoded from the p53 tumor suppressor gene in maintaining the undifferentiated state of human myeloid leukemias

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ABSTRACT

Many human myeloid leukemias represent proliferating progenitor cells that are blocked in their ability to undergo terminal differentiation. The ability to induce differentiation of these cells has been used as a strategy of cancer therapy. During our analysis of the structure and regulation of expression of the p53 tumor suppressor gene we identified a gene encoding a long non-coding RNA (lncRNA) molecule within the 10,000 bp first intron. Here we demonstrate that this novel lncRNA is expressed in undifferentiated human myeloid leukemia cells and significantly reduced during the terminal differentiation of these cells into monocytes or macrophages. Thus, we hypothesize that this lncRNA may play some role in differentiation and be required to maintain the proliferative undifferentiated state. Recently, numerous long non-coding RNA molecules have been demonstrated to function as important regulators of gene expression and shown to be involved in numerous pathways, particularly pathways dealing with cellular differentiation. Since this lncRNA, which we have named lncRNAp53int1, appears to play some role terminal differentiation of human promyelocytes, our goal is to identify the function and mechanism of action of this novel transcript. We hypothesize that it may function in a non-coding fashion as part of an uncharacterized ribonucleoprotein complex or in some other fashion to participate in the maintenance of the undifferentiated state. Our findings show that it may participate in inhibiting the expression of the p21 gene, a known negative regulator of the cell cycle. That this lncRNA is reduced in its expression during terminal differentiation of human leukemic cells is particularly intriguing in that the ability to induce differentiation of leukemic cells has been used as a therapeutic strategy for over 30 years. Differentiation therapy continues to hold promise as new targets that control differentiation are defined. A regulatory role for lncRNAp53Int1 in leukemic cell differentiation would provide us with a new and potentially powerful target with respect to therapeutics.

Contents

Abbreviations: lncRNA, long non-coding RNA; PMA, phorbol 12-myristate 13-acetate; RA, retinoic acid; RPMI, Roswell Park Memorial Medium; FITC, fluorescein isothiocyanate; HSV-TK, herpes simplex virus thymidine kinase; MMTV, mouse mammary tumor virus; EBV, Epstein Barr Virus.

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1. Introduction

Myeloid leukemias or myeloproliferative neoplasms are generally characterized by clonal expansion of cells that constitutively express proliferative signaling and regulatory pathways and often express properties similar to normal hematopoietic stems cells (Alcalay et al., 2001; Fröhling et al., 2005). Under the appropriate conditions these cells can sometimes be induced to undergo differentiation to more mature terminally differentiated and non-proliferative cell types. Consequently there are number of human myeloid leukemia-derived cell lines can be induced to differentiate into mature macrophages and undergo apoptosis by treatment with agents such as retinoic acid, phorbol 12-myristate 13-acetate (PMA), or cholinergic activation by agents such as carbachol (Lotem and Sachs, 1979; Collins et al., 1990; Carruba et al., 2003; Suzuki et al., 2009; Aydin et al., 2013; Chotirat et al., 2016). As such, these cells have been used as model systems for particular stages of monocyte differentiation (FANTOM Consortium, 2009; Suzuki et al., 2009). Understanding the mechanisms of differentiation and the blocks that occur in oncogenesis of these cell types can have important implications regarding treatments (Froghling et al., 2005).

The p53 tumor suppressor gene is one of the most frequently mutated genes identified in human cancers (Freed-Pastor and Prives, 2012; Kandoth et al., 2013). The p53 protein is responsible for a cell-cycle checkpoint that is activated after exposure to numerous DNA damaging agents or cell stressors, leading to either cell-cycle arrest or apoptosis (Oren and Bartek, 2007; Vousden and Prives, 2009; Levine and Oren, 2009; Hu et al., 2012).

A number of years ago, we identified a novel transcription unit located in the 1st intron of the p53 gene that encodes a lncRNA that we named lncRNAp53Int1 (Reisman et al., 1997; NCBI Accession U58658). This transcript has been classified as a lncRNA (Pang et al., 2005; Derrien et al., 2012; Spizzo et al., 2012; Zhao et al., 2016) and is listed as GC17M015273 in the GeneCard Human Gene Database (http://www.genecards.org) and NONHSAG020729 in NONCODE (http://noncode.org/index.php). The lncRNAp53Int1 transcript is approximately 1125 nucleotides in length, is polyadenylated, and contains no introns. Here we demonstrate that the abundance of this lncRNA is significantly reduced during differentiation and apoptosis of human myeloid leukemia cells and thus we hypothesize that its reduced expression may play a crucial role in differentiation and the loss of proliferative activity.

Our goal is to determine the function of lncRNAp53Int1, its role in maintaining pluripotency and proliferation, and the regulatory pathways in which it participates. The fact that this lncRNA is selectively reduced during terminal differentiation and the loss of proliferative capabilities of human myeloid leukemic cells, is particularly exciting because the ability to induce differentiation of leukemic cells has been used as a therapeutic strategy for over 30 years (Nowak et al., 2009; Sell, 2005; Su et al., 2014) and therefore may represent an additional target for therapeutics.

2. Materials and methods

2.1. Cell culture

The human myeloid leukemia-derived cells lines HL-60, K562, HEL, U936, as well as the human lymphoid lines IM-9, 7666, and Namalwa were grown in RPMI medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Hela and 293 cells as well as colon-derived lines COLO320 and SW837 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10%fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were cultured at 37 °C in a humidified 6% CO_2-containing incubator. Agents to induce differentiation of myeloid cells were: 20 ng/ml PMA or 2 mM retinoic acid.

2.2. Differentiation assay

Flow cytometry was used to demonstrate expression of CD11β on the cell surface and the differentiation of myeloid leukemia cells to monocyte-like cells. After treatment of cells with differentiating agents, cells were harvested and washed with PBS. 1×10^6 cells were suspended on 0.5 ml PBS and incubated in the presence of FITC-conjugated anti-CD11β antibody, washed and fractionated by flow cytometry.

2.3. Transient transfection and reporter gene assays

293 cells were grown to confluency and split to 5×10^4 cells/well in a 24 well plate. The cells were allowed to adhere overnight. The cells were co-transfected with 0.25 μg of a luciferase expression vector (pGL3 – Promega) carrying the p21 promoter, varying amounts of an expression vector (pcDNA) that expresses lncRNAp53Int1, and 50 ng of a HSV-TK renilla luciferase expression vector as an internal control. The cells were harvested 24 h later. Cellular extracts were obtained and luciferase and renilla activities were measured. All experiments were repeated at least twice and results were normalized to the renilla activity.

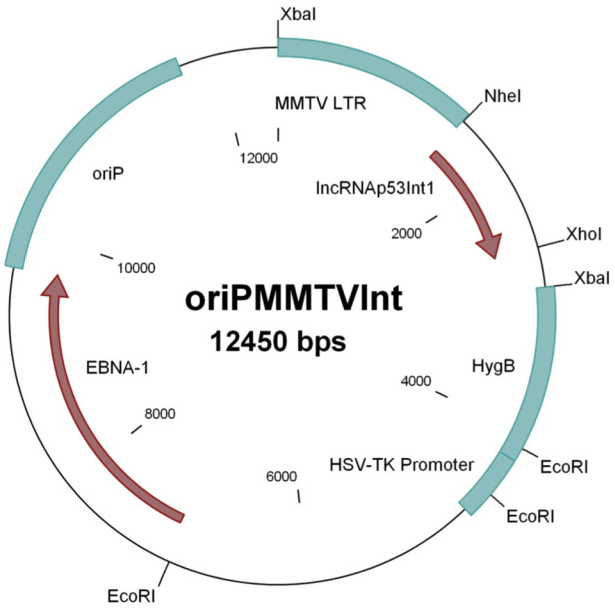

Fig. 1. EBV-based expression vector expressing lncRNAp53int1 from an MMTV (glucocorticoid-inducible) promoter. The vector carries the EBV oriP as well as the EBV EBNA-1 gene that allows this plasmid to replicate as a plasmid in human cells.