

Expression of HSATII noncoding RNA in transfected fibroblast cells

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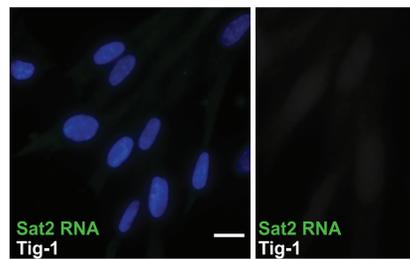


Abstract

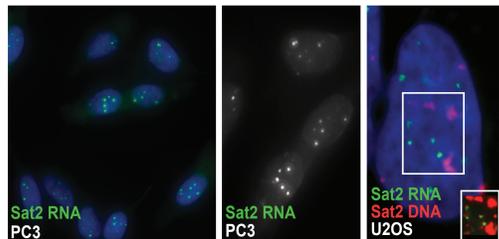
Human Satellite 2 (HSATII) is a tandemly repeated, ~26bp monomer sequence present in the pericentric heterochromatin on a subset of human chromosomes. Previously, it was demonstrated that HSATII RNA is expressed in many cancer types, producing a long non-coding RNA that accumulates in foci in cancer cells, while these transcripts are never observed in normal human cells. Further, this RNA accumulates in cancer cells in cis and binds key regulatory proteins, which are recruited to these nuclear foci. In an effort to understand the effect of HSATII RNA expression in cells that do not normally express HSATII, we created stably transfected primary human cells expressing HSATII RNA. In these stably expressing cells, we demonstrate that the HSATII expression construct is randomly integrated into the genome and the majority of cells contain a single site of integration. We observe focal accumulations of HSATII RNA in a subset of these transfected cells, demonstrating that ectopically-expressed HSATII RNA produces focal accumulations in the nucleus, with the capacity to recruit their protein binding partners and elicit changes in nuclear protein distribution.

HSATII (Sat2) expression in cancer cells

Normal Cells



Cancer Cells



Cancer Cell Line	Cell Type	Aberrant Sat 2 RNA Foci
U2OS	Osteosarcoma	+++
PC3	Prostate Adenocarcinoma	++
MCF-7	Breast Adenocarcinoma	++
HT-1080	Fibrosarcoma	++
HCC-1937	Breast Ductal Carcinoma	++
T47D	Breast Ductal Carcinoma	+
SAOS-2	Osteosarcoma	+
HEP-G2	Hepatocellular carcinoma	+
JAR	Choriocarcinoma	-
HELA	Cervical Adenocarcinoma	-
HCT	Colon Adenocarcinoma	-
MDA-MB-231	Breast Adenocarcinoma	-

Normal and non-cancerous cell lines

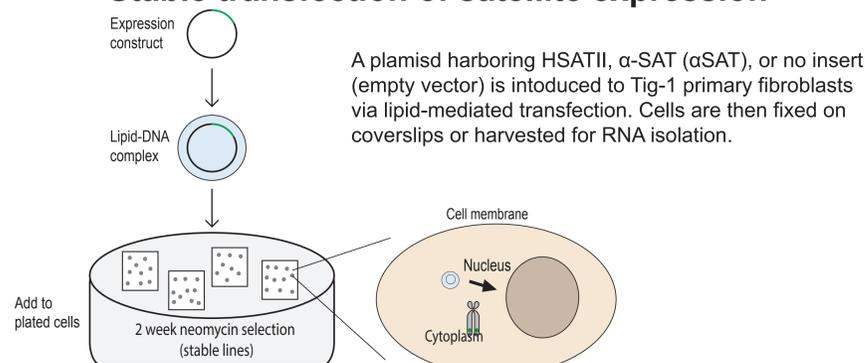
Cell Line	Cell Type	Aberrant Sat 2 RNA Foci
MCF-10A	Breast Fibrocytic Disease	-
IMR-90	Lung Fibroblast	-
WS1	Embryonic Skin Fibroblast	-
TIG-1	Fetal Lung Fibroblast	-
HFF	Foreskin Fibroblast	-
HSMM	Skeletal Myoblasts	-
HSMM	Differentiated Myotubes	-

HSATII is aberrantly expressed and accumulates in cis in cancer cell lines. Fluorescence in situ hybridization (FISH) indicates HSATII aberrant focal accumulation is detected in a wide array of cancer cell lines. No expression is detected in a panel of normal (non-cancerous) cell lines. HSATII RNA foci accumulate adjacent to their pericentric DNA locations in the interphase nucleus (bottom, far right) (Hall et al., 2017).

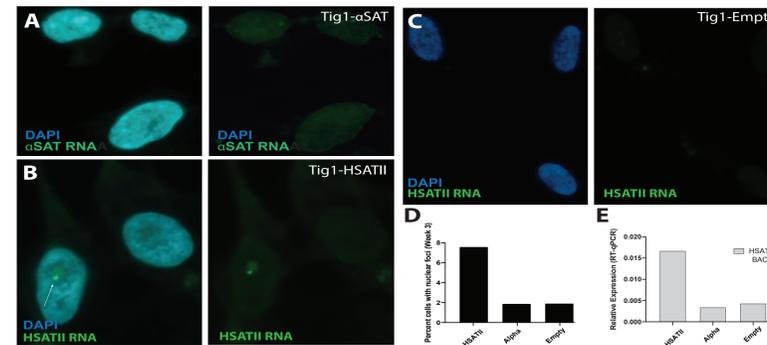
Overall Question

What are the consequences of HSATII expression?

Stable transfection of satellite expression

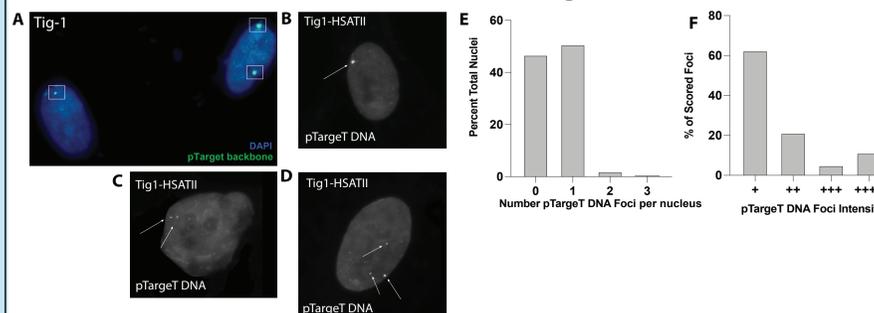


Expression of satellite RNA results in focal nuclear accumulation of HSATII RNA



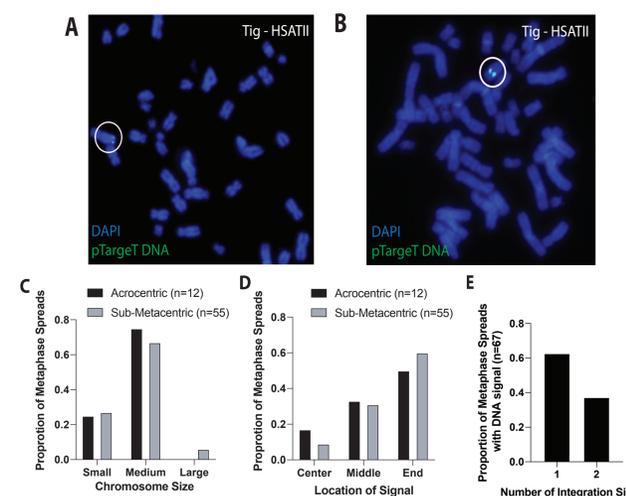
RNA FISH demonstrates that (A) α SAT RNA is primarily diffuse in the nucleus (B) HSATII RNA accumulates in distinct nuclear foci (C) No expression is detected within cells transfected with an empty vector (D) Quantification of cells with HSATII nuclear expression 3 weeks following transfection (E) RT-qPCR of HSATII RNA in transfected cell lines. Data is normalized to β -actin. Error bars show 95% confidence interval.

High efficiency integration of pTargetT vector DNA in HSATII transfected Tig-1 cells



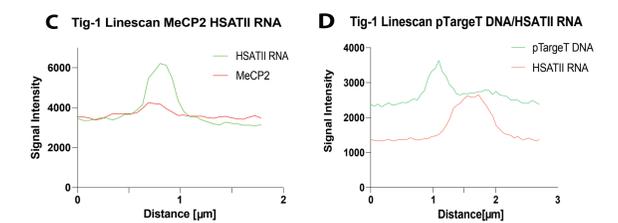
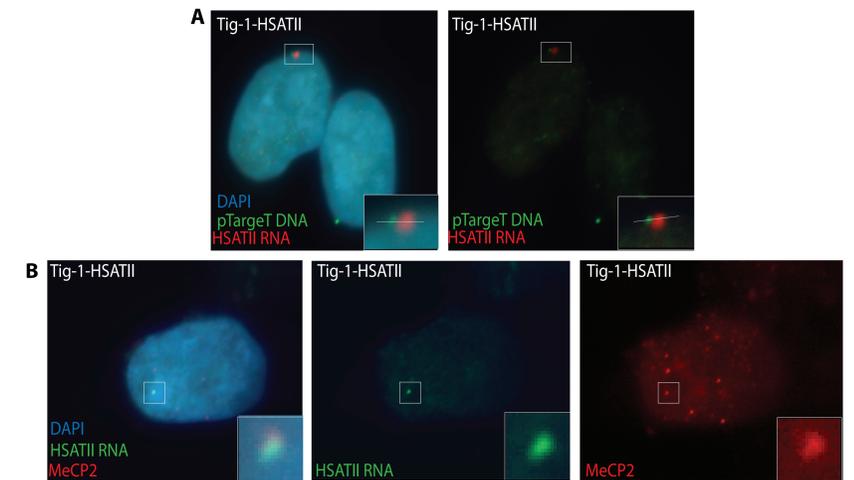
HSATII transfected nucleus with one (B), two (C), or three sites of integration (D). DNA FISH with the pTargetT backbone shows at least one site of integration in 54.96% of HSATII transfected cells (N=200) (E). Not all nuclei displayed detectable pTargetT DNA signal due to incomplete DNA hybridization efficiency. Under the same exposure time, intensity of scored foci were categorized: + (dim), ++ (easily visible), +++ (very bright), or ++++ (extremely bright, saturated pixels) (F).

pTargetT vector randomly integrates into Tig-1 chromosomes



Categorization of (C) chromosome size and (D) signal location were scored for both sub-metacentric and acrocentric chromosomes and demonstrate random integration on a variety of human chromosomes. The number of pTargetT vector DNA integration sites on HSATII transfected Tig chromosome spreads was also scored (E). Data shown is for chromosome spreads with detectable pTargetT DNA hybridization signal.

HSATII RNA accumulates adjacent to site of integration and recruits MeCP2



Dual channel linescan of inset in part A exhibit HSATII RNA and pTargetT DNA signals are adjacent and not overlapping (D). Tig-1 fibroblasts stably expressing HSATII RNA demonstrate overlapping signals for HSATII RNA and MeCP2 (B). Dual channel linescan of inset in part B exhibit HSATII RNA and MeCP2 signals overlapping (C).

Summary

We generated stable human cell lines exogenously expressing satellite sequences. We report that in a stably expressing HSATII cell line, HSATII RNA formed into distinct nuclear bodies, similar to the pattern of endogenous HSATII expression in cancer cells. HSATII expression leads to the accumulation of key regulatory proteins, such as MeCP2.

Timeline of ectopic HSATII expression

