Use of the Gene Trap Resource for Cancer-related IncRNAs to Study the Role of Malat1 in Pancreatic Cancer.

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Abstract

Texas A&M Institute for Genomic Medicine (TIGM) houses the world’s largest library of knock-out mouse ES cells and provides transgenic mice for research worldwide. Among multitude of genetic targets inactivated in these cells, there is a significant group of long non-coding RNAs (lncRNAs), the non-protein encoding transcripts longer than 200 nucleotides implicated in a variety of disease states and demonstrated their involvement in oncogenesis. Our screening of more than 18,000 clones has identified over 1,000 inactivated miRNAs including a number of IncRNAs. One such clone, D1S5416611, was used to establish a colony of homozygous mouse Metastasis-Associated-Lung-Adenocarcinoma-TranScript-1 (Malat1) mice in pure C57BL/6j genetic background. Malat1 is an IncRNA that is overexpressed in multiple cancer cell lines and tumors. The highly conserved mouse homologue of Malat1 was found to be highly expressed in hepatocellular carcinoma and now we have demonstrated that Malat1 is also pro-oncogenic in pancreatic cancer cells. Homozygous mice are viable and don’t display any gross phenotype. The effects of loss of Malat1 expression on pancreatic tumor formation will be determined.

Introduction

With the advent of high throughput genome sequencing and annotation technologies, the increasing significance of non-protein encoding RNAs (lncRNAs) in cellular homeostasis and disease has become apparent [1]. MicroRNAs (miRNAs) are small non-coding RNAs containing 21-23 nucleotides and the >1000s miRNA promoters repress gene expression, and it has been estimated that miRNAs regulate up to 50% of all mRNAs. Long non-coding RNAs (lncRNAs) contain >200 nucleotides and recent reports from the ENCODE Project Consortium have identified >2,000 lncRNAs expressed at >14,800 transcripts [2].

Metastasis-Associated-Lung-Adenocarcinoma-TranScript-1 (Malat1) is an IncRNA that is overexpressed in multiple cancer cell lines and tumors, and Malat1 expression is a prognostic factor for decreased survival of stage 1 non-small lung cancer (NSCLC). Malat1 expression is also associated with metastasis in NSCLC patients and Malat1 expression is correlated with poor prognosis (survival/recurrence/metastasis) in squamous cell carcinoma of the lung, hepatocellular carcinoma, bladder and osteosarcoma. Moreover, functional studies determined that Malat1 knockdown or overexpression indicate that Malat1 enhances cancer cell invasion, migration, invasion and epithelial-to-mesenchymal transition. Moreover, we have now demonstrated that Malat1 is also pro-oncogenic in pancreatic cancer cells. The highly conserved mouse homologue of Malat1 is also expressed in hepatocellular carcinoma [3] and we have identified Malat1 expression and function in pancreatic cancer and our proposed studies will investigate generation of IncRNA knockout mice and Malat1 mice will be crossed with transgenic mouse models expressing KrasG12D with a p53 mutation in the pancreas and 100% of these develop pancreatic tumors.

Technology

The basic gene trap vectors we have used include a reporter gene downstream of a splice acceptor sequence (Fig. 3).

Fig. 1. Gene trap vectors used in C57BL/6J library (genes shown vector): C7L, long terminal repeat; A, splice acceptor sequence; pgy, galactosidase/omega phage promoter; pCAG, CAG promoter; pA, polyadenylation sequence.

They are designed to function when inserted in an intron, to produce indirect splicing of the target gene such that all exons downstream of the insertion site are not expressed. The gene trap cassette is inserted in a retroviral vector. Retrovirus insert is a single copy per locus, with no rearrangement of flanking sequences. They have a preferential bias in CAGCG/C of genes, often centered on the intron 45F, and the splice acceptor sequence we use do not appear to be bypassed by the RNA-splicing machinery. As a result, the majority of the mutations generated using our gene trap vectors are predicted to lead to null alleles.

References


Results

Expression of Malat1 in pancreatic cancer cells

Our studies show that Malat1 is expressed in pancreatic cancer cells and can be detected in serum from pancreatic cancer patients, however with the reported high expression of Malat1 in pancreatic tumors compared to normal tumor tissues [3].

We also investigated the effects of Malat1 knock down on both cell viability and migration assays (Fig. 3A), and the results (Fig. 3A) show that loss of Malat1 in Panc1 and Panc2Cl cells resulted in significant inhibition of cell proliferation compared to cells transfected with a control oligonucleotide (siLamin). Thus, Malat1-1 and Malat1-1 regulated genes play a role in pancreatic cancer cell growth. Malat1-1 knockout mice were born at the expected Mendelian ratio by RNAi and also significantly decreased transwell migration in Panc2Cl and Panc1 cells using a Boyden chamber assay (Fig. 3B).

Generation of Malat1 knockout

The bioinformatics efforts have revealed that more than 18,000 ES cell clones from our collection have insertions which appear to inactivate 1,000 unique long nonRNAs (those greater than 200bp). Included in these are lncRNAs such as Malat1, Tisx and Avi. TIGM C57BL/6J ES cell clone D1S5416611 was found to carry mutation in Malat1 (Fig. 4) and was chosen for further study. The clone was expanded and the genomic sequence surrounding the gene trap insertion site was determined as follows (the insertion site is denoted with an asterisk *). The IS144611 clone was cloned to establish a colony of homozygous mutant Malat1 mice in pure C57BL/6J genetic background. Mutant mice were generated using standard procedures. In short, a mutant ES cell clone was expanded and microinjected into albino B6 blastocysts to generate germline chimeras. Those were bred to C57BL/6j females for germline transmission of the mutant Malat1 allele. The IS144611 copy of the genomic insertions site and for the vector (Fig. 2).

Table 2 - Schematic representation of the mutated locus markers: D1S5416611-C, D1S5416611-D and D1S5416611-B. The downstream region is the primer used in germline genotyping. Malat1-1 and Malat1-2 are primers used to confirm knockout via RT-PCR.

Fig. 3. Transwell migration assay showing reduced migration of Panc1 and Panc2Cl cells following knockdown of Malat1 by siRNA.

Fig. 4. Schematic representation of the mutated locus markers: D1S5416611-C, D1S5416611-D and D1S5416611-B. The downstream region is the primer used in germline genotyping. Malat1-1 and Malat1-2 are primers used to confirm knockout via RT-PCR.

Overall, our preliminary data indicates that genomic localization of the gene trap insertions from TIGM library along with mapping of the non-coding transcriptome can serve as a very effective tool to segment out a collection of functional mutations in thousands of mouse IncRNAs that, in turn, can be used to produce a repository of novel mutant mouse models for various research areas.

Conclusions and Future Directions

TIGM maintains the world’s largest library of stable mouse knockout embryonic stem (ES) cells in the C57BL/6 background, with a total of over 350,000 clones representing more than 10,000 unique protein-coding genes. The library has been analyzed to identify over 1,000 inactivated miRNAs including a number of IncRNAs. As a proof of concept, we have successfully established a mutant mouse line carrying a homozygous mutation in Malat1, an IncRNA involved in several different types of cancer, including pancreatic. Future efforts will include continuing analysis of the TIGM ES cell collection to discover and verify additional inactivated IncRNAs. Clones with these mutations will be made easily accessible to the scientific community and can be found on the TIGM website using text or sequence searching tools. TIGM is also planning to produce more mutant mouse lines with disrupted IncRNAs of the highest scientific value, most of which will include potential cancer targets, and make them available to the scientific community. Also, we have demonstrated that Malat1 is pro-oncogenic in pancreatic cancer cells. Homozygous mice are viable and don’t display any gross phenotype; therefore, in order to investigate the role of Malat1 in pancreatic cancer, these mutant mice are now being crossed with a transgenic mouse model expressing KrasG12D and carrying the p53 mutation that effectively develops pancreatic tumors. The effects of loss of Malat1 expression on pancreatic tumor formation will be determined.