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14. ABSTRACT tRNA reads mRNA codons in translation and is essential for protein synthesis. Like mRNA, tRNAs are also under stringent cellular control. Our proposal aims to determine the feasibility of using tRNA expression as biomarkers for breast cancer type and progression, and how tRNAs are used to regulate gene expression in breast cancer cells. We found that the expression of both nuclear and mitochondrial-encoded tRNAs can indeed be useful as possible biomarkers for breast cancer types. tRNA expression also changes in discernable patterns upon treatment of drugs that target breast cancer cells. Furthermore, specific patterns for a subset of tRNAs are found that suggest a previously unknown link between tRNA and cell growth through signal transduction pathways. These results demonstrate that studies of tRNA and breast cancer biology will be useful in understanding breast cancer type and progression and may lead to new drug targets for breast cancer treatment.					
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INTRODUCTION:

tRNAs read mRNA codons in translation and are essential for protein synthesis. Like mRNA, the concentration and the identity of tRNAs are also under stringent cellular control. Our project aims to determine whether tRNAs serve as biomarkers for breast cancer cells and whether tRNAs in normal and breast cancer cell lines control the expression of selected genes that are important for the tumorigenic process. Finding tRNA as biomarkers could open up a new and so far under-appreciated avenue for detecting the type and stage of breast cancer progression. Identification of tRNAs that regulate tumor development and progression could produce targets for a new class of cancer drugs.

BODY:

Task 1: Development of a “second-generation” tRNA microarray capable of studying single-nucleotide differences in human tRNA. → **Completed.**

Our “first generation” microarray identifies tRNA based on hybridization alone and could not distinguish tRNAs with less than 8 nucleotide differences in their sequences ((1), Fig. 1A). To enable detection of single-base difference between tRNAs, we developed a “second-generation” method based on the principle of chemical ligation (Fig. 1B). Enzymatic ligation methods are commonly used to probe single-base differences in DNA sequences, but are inefficient on RNA templates and can result in poorly reproducible data due to slight changes in reaction conditions (2, 3). An appealing alternative to enzymatic ligation is the non-enzymatic autoligation, in particular 3’phosphorothioate-5’iodide autoligation chemistry (Fig. 2A). This reaction proceeds efficiently at moderate temperatures, yields high discrimination against point mutations, and works well on DNA templates (4, 5).

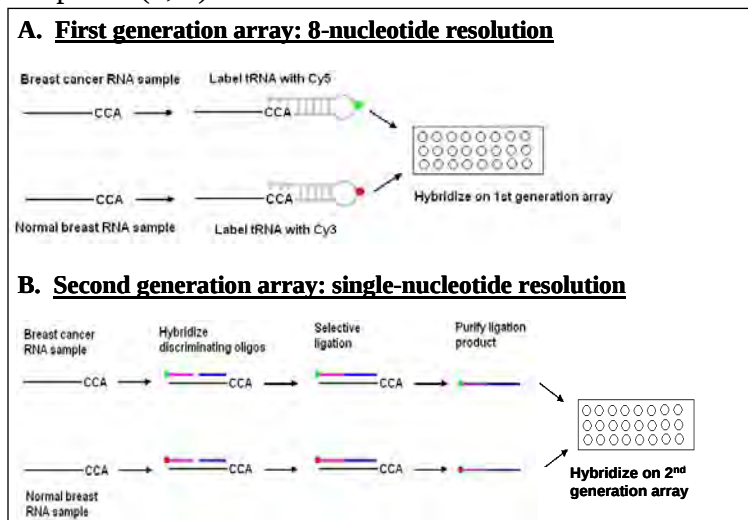


Fig. 1 – tRNA arrays for detection and quantitation of all tRNA isoacceptors. (A) First generation array. All tRNAs in a human total RNA sample are 3’CCA-labeled by ligation to a fluorescent oligo, and then hybridized onto complementary probes on the array. (B) Second generation array. Two probes complementary to the target tRNA are added to the sample. One of the probes is fluorophore-labeled for subsequent detection. Selective chemical ligation allows discrimination of single-nucleotide differences in tRNAs. After enzymatic degradation of the tRNA, the ligation product is hybridized onto complementary probes on the array.

The development of the second-generation method took place in two stages. In the first stage, a model 30-mer RNA was used to determine whether 5’iodide and 3’thiophosphate DNA oligonucleotide probes prefer one base over the other in ligation (Fig. 2B). Ligation yield is highly sensitive to the presence of a mismatch at the ligation junction, so that ligation proceeds efficiently only when the DNA probes are fully complementary to the RNA template at the ligation junction (Fig. 2C). This method was verified on full-length purified yeast tRNA^{Phe}. In the second stage, this autoligation chemistry was applied to the detection and quantitation of single-base differences at the first position of the tRNA anticodon. We have now synthesized and tested 3’phosphorothioate and 5’iodide probe sets for discrimination of all target tRNA isoacceptor pairs for the subsequent

microarray analysis, allowing detection and quantitation of all 49 tRNA isoacceptors in a human cell.

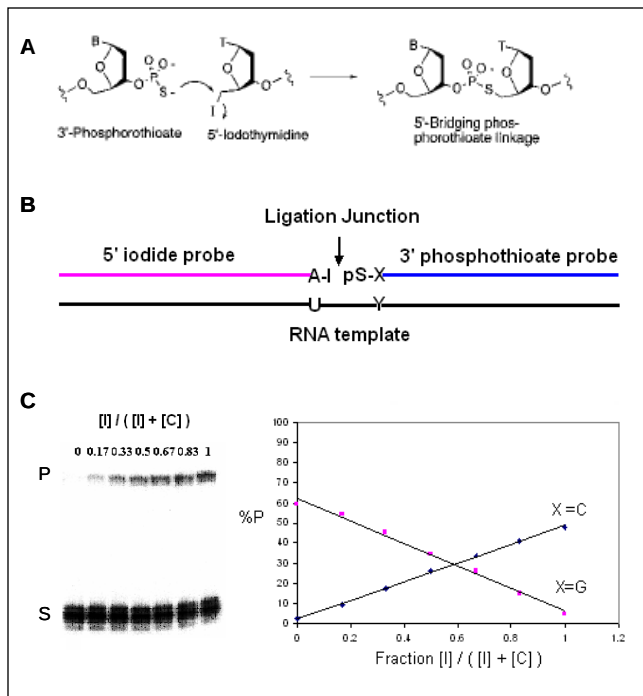


Fig. 2 – Chemical ligation detects and quantifies single-base differences at the tRNA anticodon.

(A) Chemical DNA autoligation involving 5'-iodide and 3'-phosphorothioate groups. (B) Oligonucleotide probes for detection of single-base differences in a tRNA, e.g. at the first position of the anticodon. The 5' iodide probe and the 3' phosphorothioate probe are complementary to the RNA template. The ligation junction is between a conserved U and the first position of the anticodon. (C) Ligation reactions using a defined mixture of model RNA 30-mers with Inosine (I) or C at the ligation junction. The thiophosphate probe has X = C, and prefers I over C. P = ligation product, S = unreacted probe. (D) The percent product from gel analysis of ligation reactions plotted versus the fraction of inosine-model template in the reaction. Similar results are obtained for U/C and I/C model templates.

Task 2: Development of a “third-generation” method capable of studying the expression of >90% of human tRNA species. → **In development.**

After the successful development of the “second-generation” microarray method (task 1), we realized that the most effective way for completing task 2 is to apply the newly available, high throughput DNA sequencing method (e.g. Solexa technology by Illumina). This new technology offers a much higher data output at a significantly reduced cost. We will continue to work on this in the second year of the project.

Task 3: Evaluate the usefulness of tRNA expression pattern as biomarkers → **In progress.**

To gain an initial insight into tRNAs and breast cancer, we measured tRNA abundance levels in four breast cancer cell lines relative to one normal breast cell line using our first generation microarray. These breast cancer cell lines were selected to cover a range of tumor types, tissue sources, and molecular signatures (Table 1). For nuclear encoded tRNAs, all breast cancer cell lines exhibit a two- to three-fold increase in global tRNA levels relative to the normal breast cell line (Fig. 3A). This general increase in tRNA expression likely reflects a higher rate of translation and cell growth, and suggests that increased tRNA levels correlate with cell proliferation. However, not all tRNAs increase equally, as seen when tRNA abundance values are normalized to the median value for each cell line (Fig. 3B and 3C). This selectivity leads to tRNA expression patterns that are markedly different not only between breast cancer and normal breast cell lines, but also among breast cancer cell lines. Though our set of selected cell lines is too small to draw definitive conclusions at this time, these distinct tRNA profiles may reflect the unique molecular signature and physiological properties of each cell line.

The global level of mitochondrial-encoded tRNAs varies more widely than nuclear-encoded tRNAs. Additionally, the patterns of individual mitochondrial-encoded tRNAs do not follow those of the nuclear encoded tRNAs (Fig. 3A and 3C). This result suggests that distinct signatures may be observed for tRNAs present in different cellular compartments, increasing the feasibility of using tRNA profiles as biomarkers.

Table 1 – Breast cancer and normal breast cell lines selected for tRNA profiling. All cell lines were obtained from the ATCC cell collection. ER = estrogen receptor, PR = progesterone receptor

Cell Line	ER	PR	Tumor Type	Tissue Source	Tumorigenic
MCF10A	-	-	Non-tumor	Normal mammary gland	No
BT-474	+	+	Invasive ductal carcinoma	Primary	Yes
MCF7	+	+	Invasive ductal carcinoma	Metastasis – Pleural effusion (lungs)	Yes
MDA-MB-231	-	-	Adenocarcinoma	Metastasis – Pleural effusion (lungs)	Yes
MDA-MB-436	-	-	Invasive ductal carcinoma	Metastasis – Pleural effusion (lungs)	Yes

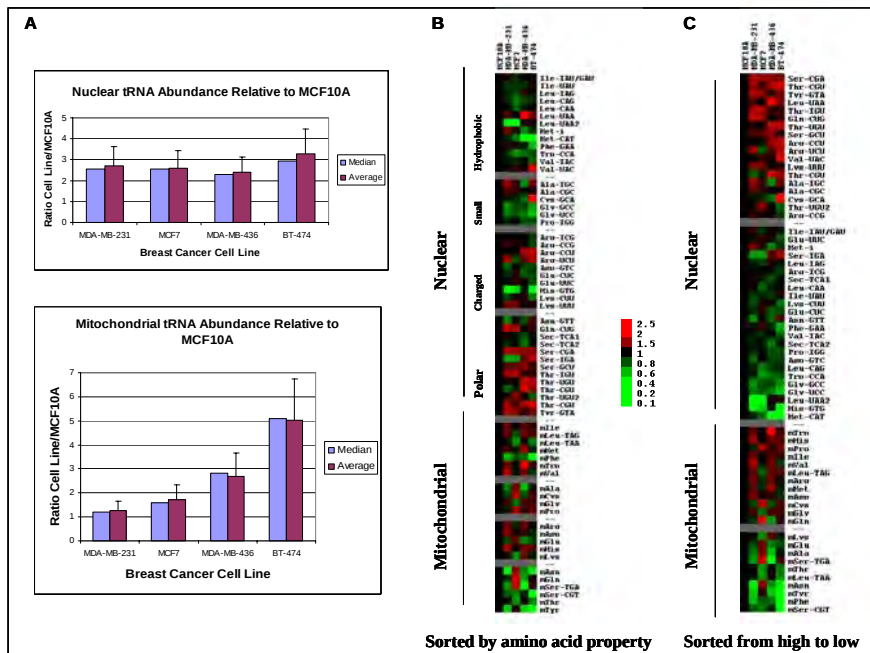


Fig. 3 – tRNA expression profiles of four breast cancer cell lines relative to a normal breast cell line. (A) Global tRNA levels. Nuclear- and mitochondrial-encoded tRNAs are presented separately. (B and C) tRNA abundance shown as TreeView images after normalization to the median value from (A). Green indicates a decreased level, red an increased level, and black an unchanged level relative to MCF10A. Data are grouped according to amino acid property (B) and from high to low (C). Many highly expressed tRNAs in breast cancer lines code for ser, thr and tyr-tRNAs – hallmark of protein phosphorylation and signal transduction.

Task 4: Identify correlations between tRNA and mRNA expression: → **In progress.**

In addition to comparing the tRNA abundance in normal versus breast cancer cell lines, we also measured changes in tRNA abundance for two breast cancer lines upon treatment with taxols using our first generation arrays (Fig. 4). Our result clearly shows a change in tRNA abundance in response to these drugs. Once the same tRNA measurements using our newly developed second-generation microarrays are performed, we will finish the computational analysis to seek for possible tRNA-mRNA correlations.

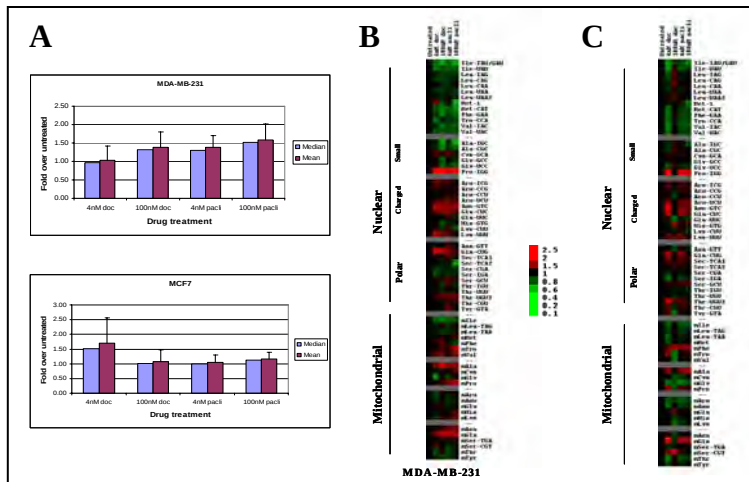


Fig. 4 – Changes in tRNA expression of two breast cancer cell lines upon drug treatment. (A) Global tRNA levels.

Nuclear- and mitochondrial-encoded tRNAs are presented separately. (B and C) tRNA abundance data shown as TreeView images after normalization to the median value from (A). Green indicates a decreased level, red an increased level, and black an unchanged level relative to untreated cells. Data are grouped according to amino acid property (B) and from high to low (C).

Task 5: Identify correlations between tRNA and active protein synthesis: → In development.

This task awaits the completion of task 4 in order to select specific gene targets for protein expression studies.

KEY RESEARCH ACCOMPLISHMENTS:

- Developed a new method that detects and quantifies tRNA at single nucleotide resolution.
- Determined the feasibility of using tRNA expression as possible biomarkers for breast cancer cells.
- Determined that tRNA expression in breast cancer cells changes in discernable patterns upon drug treatments.
- Discovered that tRNAs coding for phosphorylated amino acid show most drastic change in breast cancer cells as compared to non-tumor breast cells, suggesting a possibly new mechanism for cell signaling.

REPORTABLE OUTCOMES:

- Poster presentation at the Era of Hope meeting, June 2008.
- Chemical synthesis of numerous oligonucleotides with non-commercially available modifications.

CONCLUSION:

Our results show for the first time that tRNAs are good candidates as molecular biomarkers for breast cancer cells. Both nuclear and mitochondrial-encoded tRNAs show distinct patterns in different breast cancer cells with varying tumorigenic characteristics. Applying higher resolution tRNA studies using our “second-generation” microarrays and high-throughput sequencing technologies, the likelihood is high that tRNAs will become a valuable class of biomarkers for breast cancer type and progression.

Another important aspect of our tRNA study is to discover the possibility that altering tRNA expression in breast cancer may lead to changes in cellular behavior. Because this area is practically not being studied prior to our work described here, using tRNAs as means to alter cell biology may

lead to the identification of certain tRNAs or their associated protein enzymes as potential new drug targets for breast cancer.

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