Human Cancer Long Non-Coding RNA Transcriptomes

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Abstract

Once thought to be a part of the 'dark matter' of the genome, long non-coding RNAs (IncRNAs) are emerging as an integral functional component of the mammalian transcriptome. LncRNAs are a novel class of mRNA-like transcripts which, despite no known protein-coding potential, demonstrate a wide range of structural and functional roles in cellular biology. However, the magnitude of the contribution of IncRNA expression to normal human tissues and cancers has not been investigated in a comprehensive manner. In this study, we compiled 272 human serial analysis of gene expression (SAGE) libraries to delineate IncRNA transcription patterns across a broad spectrum of normal human tissues and cancers. Using a novel IncRNA discovery pipeline we parsed over 24 million SAGE tags and report IncRNA expression profiles across a panel of 26 different normal human tissues and 19 human cancers. Our findings show extensive, tissue-specific IncRNA expression in normal tissues and highly aberrant IncRNA expression in human cancers. Here, we present a first generation atlas for IncRNA profiling in cancer.

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Introduction

Genome instability and mutation are a hallmark of cancer [1]. Genetic and epigenetic changes result in aberrant expression of protein-coding genes and many classes of non-coding RNAs (ncRNAs), including microRNAs (miRNAs). MiRNAs have proven to be major players in human carcinogenesis, despite comprising only a small fraction of ncRNAs [2].

Once thought to be the 'dark matter' of the genome, ncRNAs have emerged as an integral component of the mammalian transcriptome [3,4,5]. These enigmatic molecules are defined by lack of protein-coding sequence, yet can play both structural and functional roles in the cell [6,7]. NcRNAs can been grouped into two major classes, the small ncRNAs, which include miRNAs and other non-coding transcripts of less than 200 nucleotides (nt), and the more recently described lncRNAs, which range from 200 nt to >100 kilobases (kb) [8].

LncRNAs can be intergenic, intronic, antisense or overlapping with protein-coding genes or other ncRNAs [9,10,11,12]. The known repertoire of lncRNA functions is rapidly expanding – with demonstrated roles as mediators of mRNA decay [13], structural scaffolds for nuclear substructures [14,15], as host genes for miRNAs [16,17], and as regulators of chromatin remodeling [18,19,20,21] – even though the functional identities of many lncRNAs have yet to be uncovered [6,7,22]. Recently, human cancers have been described to have altered expression of satellite repeats [23], transcribed ultra conserved regions (T-UCRs) [24], and antisense transcripts [25]. Beyond expression changes, accumulating evidence indicates aberrant expression of lncRNAs may play an important functional role in cancer biology [26,27,28]. The well-studied HOX antisense intergenic RNA (HOTAIR), for example, is highly expressed in breast cancers and breast cancer metastases and plays a role in retargeting chromatin remodeling complexes [29]. Similarly, high expression of the nuclear speckle associated lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALATI) modulates alternative splicing and has been associated with metastasis and poor outcome in patients with lung cancer [30,31]. While these examples are intriguing, the extent of the contribution of differential lncRNA expression to human cancer is currently unknown.

With a conservative estimate of 23,000 lncRNAs in the human genome, these transcripts rival the \sim 20,000 protein-coding genes [5,11,32,33]. Over the past two decades, microarray profiling has generated a wealth of information on protein-coding gene expression patterns in human cancers. However, as lncRNA specific probes are underrepresented on commercial microarrays used in cancer transcriptome profiling, these data do not apply to ncRNAs. Global sequencing of RNA populations is a new approach used to profile RNA expression levels that will capture the extent of lncRNA expression. Recently, genome-wide ncRNA expression profiles were determined in 11 samples representing different types of human tissues [34].

One sequence-based method for enumerating the abundance of polyadenylated transcripts is SAGE [35]. As many lncRNAs themselves are polyadenylated, lncRNA transcript levels can be deduced by way of direct enumeration of corresponding sequence tags using SAGE technology. In fact, two antisense lncRNAs were discovered using a SAGE-based method [25]. Since the invention of SAGE technology in the mid 1990s, numerous SAGE libraries representing a diversity of human and mouse, normal and malignant tissues and cell lines have become publically available [36]. Of the 755 human SAGE libraries in the Gene Expression Omnibus (GEO) database, ~276 include SAGE libraries derived from human cancers or dysplasias [37].

In this study, we compiled 272 human SAGE libraries to delineate lncRNA transcription patterns across a broad spectrum of human tissues and cancers. Using a custom lncRNA discovery pipeline, we parsed over 24 million SAGE sequence tags to deduce (1) the specific lncRNA expression patterns in 26 human tissues and discovered ubiquitously expressed as well as tissue specific lncRNAs, and (2) the aberrant expression patterns of lncRNAs in 19 human cancers.

Results

Assembling human SAGE libraries of normal and cancer tissues

A total of 1,824 SAGE libraries (in short SAGE, long SAGE and SAGE-seq format) of human and non-human origins are publically available via GEO. To explore lncRNA expression in the broadest range of human tissue types and cancer types, we downloaded 360 GEO accessioned human short SAGE libraries comprised of libraries curated by the Cancer Genome Anatomy Project (324 libraries) and lung tissue and cancer datasets (36 libraries) (Table S1). Individual libraries were filtered for sequence depth, retaining only those libraries with >50,000 raw tags, to provide 272 SAGE libraries for analysis using our lncRNA discovery pipeline (Table S2). The 272 SAGE libraries are comprised of a total of 24,436,076 raw sequence tags with an average raw tag count of 90,212 per library. Collectively, the libraries spanned 26 normal human tissue types, including 19 human cancer types, and 9 tissue types derived from cell line libraries (Figure 1, Table S3).

Long non-coding RNA discovery pipeline

To generate lncRNA expression profiles, we developed a lncRNA discovery pipeline to map tag-to-lncRNA matches (Figure 2). A SAGE tag expression matrix was constructed from all unique tags (n = 716,330) identified within the dataset of 272 libraries. Unigene mapped and unmapped SAGE tags (n = 269,785 and n = 446,545, respectively) were separated into distinct expression matrices which were subsequently filtered to retain only those tags with at least 2 raw tag counts in 3 or more SAGE libraries. Using SAGE Genie to assign gene identifiers to the Unigene IDs, 263 of the 61,054 filtered tags with corresponding Unigene IDs mapped to known lncRNAs, and 15,773 tags either lacked gene names or had ambiguous annotations (e.g. transcribed loci, cDNAs, hypothetical genes). Based on the absence of confirmed association with known genes, these 15,773 tag-to-Unigene ID matches were considered as candidate lncRNA tags.

The 15,773 Unigene tags with ambiguous gene identifiers were combined with the 17,816 unmapped, filtered tags for a total of 33,589 SAGE tags with the potential to generate tag-to-lncRNA matches. Using SeqMap, we mapped 7,040 of the 33,589 tags to lncRNA sequences from the reference lncRNA list (Table S4). The proportion of tag-to-lncRNA matches is consistent with the fact that our reference list of 9,891 lncRNAs represents only a portion

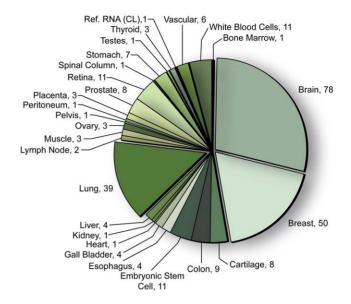


Figure 1. Tissue-type distribution of the 272 SAGE libraries with a minimum raw tag count of 50,000. (CL) indicates one SAGE library that was generated from a mixture of human cell lines. doi:10.1371/journal.pone.0025915.q001

of the estimated 23,000 lncRNAs in the genome [33]. The remaining tags that do not map to lncRNAs from our reference list may represent antisense transcripts to protein-coding genes or other ncRNAs which were filtered.

Of the 7,040 lncRNA tag matches, 3,831 mapped in the forward orientation, while 3,209 mapped in the reverse direction. In SAGE, tags matching transcript in the forward orientation are likely derived from that transcript, while tags matching in the reverse orientation are not. This is true regardless of whether the gene is normally transcribed from the plus or minus DNA strand. In this study, we were interested in the expression profiles of a curated set of lncRNAs, rather than novel gene discovery. As reverse tag matches do not corroborate the expression of the lncRNAs described herein, these tags were excluded from further analysis.

The 3,831 tags newly mapped to lncRNAs were combined with the 263 tags identified from Unigene mapping for a total of 4,094 tags uniquely mapping to lncRNAs. Where multiple tags mapped to a distinct lncRNA, the tags were collapsed by summing the tag counts to capture all transcript variants and isoforms. The end result was a lncRNA expression matrix consisting of 2,649 distinct lncRNAs (Tables S5 and S6). The lncRNAs with the highest expression were detectable in the majority (>90%) of the 272 libraries (Table 1). These included characterized examples such as nuclear paraspeckle assembly transcript 1 (*NEAT1*) and growth arrest-specific 5 (*GAS5*).

Long non-coding RNA expression profiles in normal human tissues

Of the 272 SAGE libraries, 72 represented normal human tissues. Expression of lncRNAs was detected in all tissue types, although the number of unique lncRNAs detected varied considerably (Figure 3A). On average, there were 145 distinct lncRNAs with a mean tags per million (TPM) of 20 detected in each tissue. Tissues such as lymph node and gall bladder showed the highest number of distinct lncRNAs, while the lowest numbers of distinct lncRNAs were found in the muscle and liver.



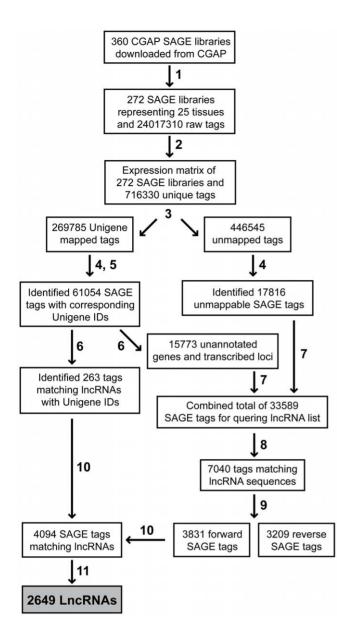


Figure 2. LncRNA discovery pipeline using SAGE analysis. Numbers indicate programs or filtering steps as follows: (1) filtering to retain only those libraries with a minimum of 50,000 raw tag counts, (2) identifying unique SAGE tags and constructing SAGE tag expression matrix, (3) mapping SAGE tags to Unigene IDs using SAGE Genie mapping files, (4) filtering lists to retain only tags with ≥ 2 raw counts in a \geq 3 of 272 libraries, (5) determining gene identity using SAGE Genie, (6) separating Unigene tags mapping to IncRNAs and ambiguous transcripts, (7) pooling ambiguous tags and unmapped tags, (8) mapping sequence tags to the reference list of 9,891 IncRNAs using SegMap, a tag-to-gene mapping program, (remaining tags may map to unannotated IncRNAs or antisense transcripts not included in our reference list) (9) filtering tag matches for strand sense, (10) pooling forward mapping tags and tags determined from Unigene, and (11) confirming tag-to-IncRNA matches and summing tag counts for IncRNAs with multiple tag matches. A complete list of IncRNAs is provided as Table S5 and tag-to-IncRNA matches are provided as Table S6

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We next focused on these libraries to determine whether tissuespecific lncRNA expression profiles could be generated (Table S7). Figure 4A shows the top 20 most highly expressed lncRNAs

Table 1. The eleven most highly expressed lncRNAs detected in >90% of the 272 SAGE libraries.

Gene Name	Ensembl Gene	Chr	Start (bp)	End (bp)	Strand
MALAT1	ENSG00000251562	11	65265233	65273940	1
GAS5	ENSG00000234741	1	173833038	173838020	-1
NEAT1	ENSG00000245532	11	65190245	65213011	1
NCRNA00188	ENSG00000175061	17	16342289	16367300	1
RP11-425M5.7	ENSG00000225759	20	36247700	36251521	-1
SNHG6	ENSG00000245910	8	67833919	67838633	-1
SNHG5	ENSG00000203875	6	86386725	86388451	-1
SCAND2	ENSG00000176700	15	85174682	85185695	1
AC104759.1	ENSG00000246638	15	31685046	31696932	1
AC002472.9	ENSG00000230513	22	21356175	21364631	1
AC090937.2	ENSG00000225733	3	14961854	14989931	-1

Also see Table S5.

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detected in the panel of normal tissues. Distinct lncRNAs detected at high expression levels in normal tissues included those characterized in the literature such as NEAT1, GAS5 and Xinactive-specific transcript (XIST). However, at least half of the highly expressed lncRNAs are novel and currently uncharacterized. To confirm the lncRNA expression profiles, we queried the expression patterns of the most highly expressed lncRNAs using RNASeq data from the Illumina Human BodyMap 2.0 project. This data was recently added to Ensembl release 62 and is presented as an optional track. Of our most highly expressed lncRNAs, the majority were widely expressed in the tissue samples from the Illumina dataset, consistent with our findings (Table S8, Figures S1 and S2). Concurrently, lncRNA expression was also found to be highly variable, with each human tissue having a unique lncRNA expression pattern (Figure 4B). Intriguingly, a number of lncRNAs were expressed in a tissue-exclusive manner (Figure 3B).

Long non-coding RNA expression profiles in human cancers

Aberrant protein-coding gene expression is well described in cancer. However, aberrant expression of ncRNAs, including miRNAs and lncRNAs, has only recently been associated with this disease [2,26,27,38]. To delineate lncRNA expression profiles associated with human cancers, we created a human cancer expression matrix based on 167 cancer SAGE libraries included in our dataset (Table S9). For the lung cancer dataset, metaplasia, dysplasia and inflammatory tissues were excluded from analysis as these represent precancerous stages [39,40]. Figure 5A shows the top 20 most highly expressed lncRNAs across the profiled cancers. Like the normal tissues, lncRNA expression in human cancer was also found to be highly variable (Figure 5B).

Human cancers demonstrate significantly altered IncRNA expression patterns

To determine the extent of differential lncRNA expression in human cancer, we created three expression matrices for each breast, brain and lung cancer which included a minimum of five normal and five cancer SAGE libraries (Table S10). The breast, brain and lung lncRNA expression matrices were independently sorted for significant and differentially expressed lncRNAs (p-

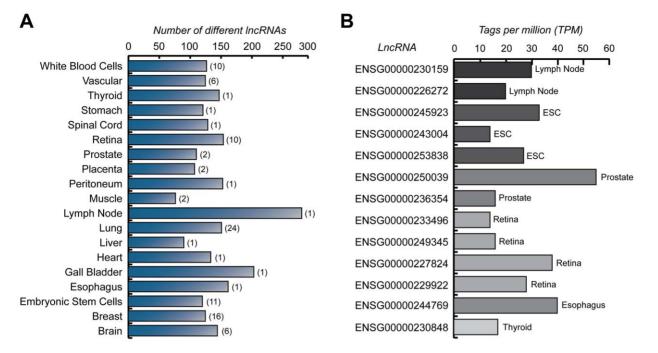


Figure 3. Distribution and levels of IncRNA expression in normal human tissues. (A) Number of distinct IncRNAs expressed in normal human tissues, white blood cells and embryonic stem cells with a minimum average TPM of 20. The values in brackets indicate the number of SAGE libraries for each tissue. (B) Examples of IncRNAs detected exclusively in a single normal human tissue or in embryonic stem cells (ESC) with a minimum expression level of 10 TPM. For tissues with two or more libraries, the TPM values were averaged. LncRNAs without names are labeled with an Ensembl ID.

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value < 0.05, ≥ 2 -fold expression change based on a nonparametric permutation test [41]). In each type of cancer, we found at least 200 lncRNAs to have significant differential expression based on these criteria (Figure 6A). Intriguingly, there was overlap between the lncRNAs that were differentially expressed in each tissue (Figure 6B), including 8 lncRNAs that were differentially expressed in all three cancers (Table 2). The ten most up- and down-regulated lncRNAs for each cancer are found in Table S11.

Chromosomal distribution of long non-coding RNAs

We constructed a distribution plot to determine the chromosomal distribution of the 9,891 lncRNA genes in our lncRNA reference list (Table S3). The lncRNAs are distributed throughout the genome and are present on every chromosome (Figure 7). Protein-coding genes and miRNAs appear to share a similar chromosome distribution (Spearman correlation p>0.05, Figure S3A). However, the chromosome distribution of lncRNAs did not correlate with either protein-coding genes or miRNAs (Spearman correlation p<0.05, Figures S3B, S3C).

Discussion

In recent years, the concept of the functional genome has been re-written to include a multitude of newly discovered classes of ncRNA transcripts [42,43,44,45]. Although the functional significance of long non-coding RNAs has long been recognized [46,47], the abundance and scale of lncRNA expression changes in cancer is just beginning to come to light. For this reason, charting the transcriptional landscape of lncRNAs across human tissue and cancer types is a key step in understanding lncRNA functional significance in cancer.

Here, we present the first multi-tissue, cross-cancer lncRNA expression profiling study. Large-scale expression profiling datasets, such as SAGE, represent a valuable resource for investigating the expression pattern of polyadenylated lncRNAs. While this approach excludes the profiling of non-polyadenylated lncRNAs, it nonetheless facilitates the simultaneous profiling of thousands of polyadenylated lncRNAs in a wide range of human tissues and cancers. Using 272 SAGE libraries, representing 26 nonmalignant human tissues, 19 human cancer types and 9 cancer cell lines, we have produced a first generation atlas of cross-cancer lncRNA expression profiles as a resource for this fast growing area of cancer research. Current estimates of the number of lncRNAs encoded in the human genome vary widely, ranging from \sim 7,000 to 23,000 or more [7]. These estimates rival the abundance of the estimated 20,000+ protein-coding genes. Our analysis showed that lncRNAs are distributed on all 22 autosomes and sex chromosomes, yet the distribution pattern did not correlate with either protein-coding genes or miRNAs (Figure 7, Figure S3).

Examination of 72 SAGE libraries of normal human tissues revealed lncRNA expression in brain, breast, esophagus, gall bladder, heart, liver, lung, lymph node, muscle, peritoneum, placenta, prostate, retina, spinal cord, stomach, thyroid, vascular tissue, embryonic stem cells and white blood cells. We find extensive and highly differential patterns of lncRNA expression in normal human tissues (Figures 3 and 4), corroborating a previous report of tissue-specific ncRNA patterns [34]. For example, the lncRNA NCRNA00116 was highly expressed in the contractile tissues, namely heart (TPM = 349) and muscle (TPM = 399). LncRNAs ENSG00000230658 and ENSG00000235621 showed very high expression (TPM = 888) in placenta and esophagus (TPM = 820) respectively, but low or undetectable expression in other tissues, which may indicate a tissue-specific role for these transcripts. The brain-associated and putative tumor suppressor lncRNA maternally

A	Brain	Breast	Embryonic Stem Cells	Esophagus	Gall Bladder	Heart	Liver	Lung	Lymph Node	Muscle	Peritoneum	Placenta	Prostate	Retina	Spinal Cord	Stomach	Thyroid	Vascular	White Blood Cells	
	223	337	20	992	875	72	392	1446	161	250	766	1912	248	337	1387	736	992	119	234	NEAT1
	711 158	345 293	88 637	172 132	187 443	157 313	45 317	353 331	201 201	844 121	953 37	370 619	664 1216	582 293	949 183	1050 872	474 923	571 381	260 129	MALAT1 NCRNA00188
	298	684	705	106	408	157	75	345	181	74	75	354	773	175	383	872	509	626	215	GAS5
	223	693	857	145	187	229	30	143	30	371	149	209	1262	170	164	95	233	631	485	ENSG00000225759
	150 48	169 201	282 238	106 26	117 397	193 120	121 60	93 113	171 30	204 427	0	70 187	207 182	295 35	292 274	341 95	190 535	149 82	157 83	SNHG6 SNHG5
	174	131	245	13	175	108	45	46	1137	74	75	111	142	61	274	55	43	110	105	ENSG00000230513
	233	155	84	212	222	132	15	271	241	111	579	62	72	176	0	0	26	176	177	ENSG00000246638
	215	197	48	26	327 222	36	15 0	120 8	10	0	19	179 94	17	202	128	464	285 9	226	53	XIST MEG3
	677 198	196 111	44	0	140	12 12	15	209	40	46 37	187 299	132	42 40	331 134	91 91	27 95	147	259 175	142	SCAND2
	125	60	104	26	292	60	106	184	121	19	56	151	107	130	237	82	104	116	84	ENSG00000225733
	26	11	138	66	0	24	15	12	0	195	131	706	0	187	0	0	9	538	2	H19
	43 33	32	22 44	13 93	70 35	349 120	75 15	45 41	40	399 9	93 75	25 56	97 32	47 8	91 55	41 123	95 78	18 455	29 74	NCRNA00116 NCRNA00152
	61	127	50	159	117	48	45	96	131	74	206	28	0	58	55	0	17	43	93	ENSG00000250682
	39	111	246	53	70	60	15	40	50	74	37	31	142	68	37	14	112	100	51	ENSG00000224812
	45 122	62 36	82 48	53 0	47 58	120 24	75 60	62 58	50 70	148 9	19 19	86 35	79 40	50 51	73 420	82 0	104 95	62 45	64 9	ENSG00000232388 SOX2OT
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в	Brain	Breast	Embryonic Stem Cells	Esophagus	Gall Bladder	Heart	Liver	Lung	Lymph Node	Muscle	Peritoneum	Placenta	Prostate	Retina	Spinal Cord	Stomach	Thyroid	Vascular	White Blood Cells	
в	0	0	0	0	0	0	0	1	0	0	0	888	0	1	0	0	0	0	0	ENSG00000230658
в	0	0 8		0 820	0	0	0 15	1	0 10	0	0	888 13	0	1	0	0	0 35	0	0	ENSG00000230658 ENSG00000235621
в	0	0	0	0	0	0	0	1	0	0	0	888	0	1	0	0	0	0	0	ENSG00000230658
в	0 6 223 10 2	0 8 337 27 1	0 6 20 3 5	0 820 992 516 0	0 0 875 23 0	0 0 72 0 0	0 15 392 0 0	1 1446 10 16	0 10 161 0 432	0 0 250 0 0	0 0 766 0 0	888 13 1912 0 0	0 0 248 8 0	1 1 337 4 2	0 0 1387 0 18	0 0 736 14 0	0 35 992 0 0	0 0 119 13 0	0 0 234 8 0	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000177112
в	0 6 223 10 2 11	0 8 337 27 1 37	0 6 20 3 5 0	0 820 992 516 0 0	0 0 875 23 0 0	0 0 72 0 0 0	0 15 392 0 0 0	1 1446 10 16 5	0 10 161 0 432 0	0 0 250 0 0 0	0 0 766 0 0 523	888 13 1912 0 0 0	0 0 248 8 0 8	1 337 4 2 11	0 0 1387 0 18 0	0 0 736 14 0 0	0 35 992 0 0 86	0 0 119 13 0 46	0 0 234 8 0 12	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000177112 ENSG00000249381
в	0 6 223 10 2	0 8 337 27 1	0 6 20 3 5	0 820 992 516 0	0 0 875 23 0	0 0 72 0 0	0 15 392 0 0	1 1446 10 16	0 10 161 0 432	0 0 250 0 0	0 0 766 0 0	888 13 1912 0 0	0 0 248 8 0	1 1 337 4 2	0 0 1387 0 18	0 0 736 14 0	0 35 992 0 0	0 0 119 13 0	0 0 234 8 0	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000177112
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В	0 6 223 10 2 11 174 26 5 223 3	0 8 337 27 1 37 131 11 2 693 4	0 6 20 3 5 0 245 138 12 857 404	0 820 992 516 0 0 13 66 0 145 185	0 875 23 0 175 0 23 187 0	0 72 0 0 108 24 60 229 0	0 15 392 0 0 0 45 15 15 30 0	1 1446 10 16 5 46 12 16 143 5	0 10 161 0 432 0 1137 0 513 30 0	0 250 0 0 74 195 37 371 0	0 766 0 523 75 131 0 149 0	888 13 1912 0 0 0 111 706 10 209 4	0 248 8 0 8 142 0 8 1262 0	1 337 4 2 11 61 187 4 170 14	0 0 1387 0 18 0 274 0 274 0 18 164 0	0 736 14 0 0 55 0 14 95 0	0 35 992 0 86 43 9 17 233 0	0 0 119 13 0 46 110 538 2 631 5	0 0 234 8 0 12 105 2 2 485 1	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000249381 ENSG00000249381 H19 ENSG00000153363 ENSG00000153363 ENSG0000025759 ENSG00000256880
В	0 6 223 10 2 11 174 26 5 223	0 8 337 27 1 37 131 11 2 693	0 6 20 3 5 0 245 138 12 857	0 820 992 516 0 13 66 0 145	0 0 875 23 0 175 0 23 187	0 72 0 0 108 24 60 229	0 15 392 0 0 0 45 15 15 30	1 1446 10 16 5 46 12 16 143	0 10 161 0 432 0 1137 0 513 30	0 250 0 0 74 195 37 371	0 766 0 523 75 131 0 149	888 13 1912 0 0 0 111 706 10 209	0 248 8 0 8 142 0 8 142 0 8 1262	1 337 4 2 11 61 187 4 170	0 0 1387 0 18 0 274 0 18 164	0 736 14 0 0 55 0 14 95	0 35 992 0 0 86 43 9 17 233	0 0 119 13 0 46 110 538 2 631	0 0 234 8 0 12 105 2 2 485	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000177112 ENSG00000249381 ENSG00000249381 ENSG000002515363 ENSG00000153363 ENSG00000225759
В	0 6 223 10 2 11 174 26 5 223 3 2 2 3 2 0 158	0 8 337 27 1 37 131 11 2 693 4 3 1 293	0 6 20 3 5 0 245 138 12 857 404 8 57 404 8 0 637	0 820 992 516 0 13 66 0 145 185 0 0 0 132	0 875 23 0 0 175 0 23 187 0 0 0 0 443	0 72 0 0 108 24 60 229 0 12 0 12 313	0 15 392 0 0 45 15 15 30 0 15 0 317	1 1446 10 16 5 46 12 16 143 5 9 0 331	0 10 161 0 432 0 1137 0 513 30 513 30 0 30 0 201	0 250 0 0 74 195 37 371 0 9 9 278 121	0 766 0 523 75 131 0 149 0 0 0 37	888 13 1912 0 0 10 111 706 10 209 4 4 4 0 619	0 248 8 0 8 142 0 8 1262 0 8 0 8 0 0 1216	1 337 4 2 11 61 187 4 170 14 8 0 293	0 0 1387 0 18 0 274 0 18 164 0 402 0 183	0 736 14 0 0 55 0 14 95 0 14 0 14 872	0 35 992 0 86 43 9 17 233 0 17 17 0 923	0 0 119 13 0 46 110 538 2 631 5 2 631 5 2 0 381	0 0234 8 0 12 105 2 2 485 1 5 0 129	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000249381 ENSG00000249381 ENSG00000230513 <i>H19</i> ENSG00000153363 ENSG00000225759 ENSG00000225759 ENSG00000224281 ENSG00000243319 NCRNA00188
В	0 6 223 10 2 11 174 26 5 223 3 2 2 2 3 0 158 1	0 8 337 27 1 37 131 11 2 693 4 3 1 293 4	0 6 20 3 5 0 245 138 12 857 404 8 7 404 8 0 637 1	0 820 992 516 0 13 66 0 145 185 0 0 132 291	0 0 875 23 0 175 0 23 187 0 0 0 443 0	0 72 0 0 108 24 60 229 0 229 0 12 0 313 12	0 15 392 0 0 45 15 15 30 0 15 0 15 0 317 0	1 1446 10 16 5 46 12 16 143 5 9 0 331 0	0 10 161 0 432 0 1137 0 513 30 0 30 0 201 0	0 0 250 0 0 74 195 37 371 0 9 278 121 9	0 0 766 0 523 75 131 0 149 0 149 0 0 0 37 0	888 13 1912 0 0 1111 706 10 209 4 4 4 0 619 6	0 0 248 8 0 8 142 0 8 1262 0 8 0 2 2 6 0 1216 0	1 337 4 2 11 61 187 4 170 14 8 0 293 3	0 0 1387 0 18 0 274 0 18 164 0 402 0 183 0	0 736 14 0 55 0 14 95 0 14 95 0 14 0 872 0	0 35 992 0 86 43 9 17 233 0 17 233 0 17 9 233 0 923 0	0 0 119 13 0 46 110 538 2 631 5 2 0 381 5	0 0 234 8 0 12 105 2 2 485 1 5 0 129 0	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000177112 ENSG00000249381 ENSG00000249381 ENSG0000025759 ENSG00000225759 ENSG00000224281 ENSG00000224281 ENSG00000243319 NCRNA00188 ENSG00000203645
В	0 6 223 10 2 11 174 26 5 223 3 2 2 3 2 2 3 158 1 4	0 8 337 27 1 37 131 11 2 693 4 3 1 293 4 0	0 6 20 3 5 0 245 138 12 857 404 8 0 637 1 0	0 820 992 516 0 13 66 0 145 185 0 145 185 0 0 132 291 0	0 0 875 23 0 175 0 23 187 0 23 187 0 0 443 0 0 0 0	0 72 0 0 108 24 60 229 0 12 0 12 0 313 12 0	0 15 392 0 0 45 15 15 30 0 15 317 0 0 0	1 1446 10 16 5 46 12 16 143 5 9 0 331 0 0	0 10 161 0 432 0 1137 0 513 30 0 513 30 0 30 0 201 0 0 0 0	0 0 250 0 0 74 195 37 371 0 9 9 278 121 9 0	0 0 766 0 523 75 131 0 149 0 149 0 0 37 0 0 0	888 13 1912 0 0 1111 706 10 209 4 4 4 0 619 6 0	0 0 248 8 0 8 142 0 8 1262 0 8 1262 0 8 0 1216 0 0 0	1 337 4 2 11 61 187 4 170 14 8 0 293 3 3 4	0 0 1387 0 18 0 274 0 18 164 0 402 0 183	0 0 736 14 0 55 0 14 95 0 14 95 0 14 0 872 0 245	0 35 992 0 86 43 9 17 233 0 17 233 0 17 923 0 0	0 0 119 13 0 46 110 538 2 631 5 2 631 5 2 0 381 5 0	0 0 234 8 0 12 105 2 2 485 1 5 0 129 0 0 0	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000177112 ENSG00000249381 ENSG00000249381 ENSG0000025759 ENSG0000025759 ENSG0000025759 ENSG00000224281 ENSG00000242319 NCRNA00188 ENSG00000203645 ENSG00000253167
В	0 6 223 10 2 11 174 26 5 223 3 2 2 2 3 0 158 1	0 8 337 27 1 37 131 11 2 693 4 3 1 293 4	0 6 20 3 5 0 245 138 12 857 404 8 7 404 8 0 637 1	0 820 992 516 0 13 66 0 145 185 0 0 132 291	0 0 875 23 0 175 0 23 187 0 0 0 443 0	0 72 0 0 108 24 60 229 0 229 0 12 0 313 12	0 15 392 0 0 45 15 15 30 0 15 0 15 0 317 0	1 1446 10 16 5 46 12 16 143 5 9 0 331 0	0 10 161 0 432 0 1137 0 513 30 0 30 0 201 0	0 0 250 0 0 74 195 37 371 0 9 278 121 9	0 0 766 0 523 75 131 0 149 0 149 0 0 0 37 0	888 13 1912 0 0 1111 706 10 209 4 4 4 0 619 6	0 0 248 8 0 8 142 0 8 1262 0 8 0 2 2 6 0 1216 0	1 337 4 2 11 61 187 4 170 14 8 0 293 3	0 0 1387 0 18 0 274 0 188 164 0 402 0 183 0 0 183 0 0 0	0 736 14 0 55 0 14 95 0 14 95 0 14 0 872 0	0 35 992 0 86 43 9 17 233 0 17 233 0 17 9 233 0 923 0	0 0 119 13 0 46 110 538 2 631 5 2 0 381 5	0 0 234 8 0 12 105 2 2 485 1 5 0 129 0	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000177112 ENSG00000249381 ENSG00000249381 ENSG0000025759 ENSG00000225759 ENSG00000224281 ENSG00000224281 ENSG00000243319 NCRNA00188 ENSG00000203645
В	0 6 223 10 2 11 174 26 5 223 3 2 2 3 2 0 158 1 4 32 677 6	0 8 337 27 1 37 131 11 2 693 4 3 1 293 4 0 26 196 5 5	0 6 20 3 5 0 245 138 12 857 404 8 0 637 1 0 637 1 1 0 18 5 5 5	0 820 992 516 0 0 13 66 0 145 185 0 145 185 0 0 132 291 0 0 0 0	0 875 23 0 0 175 0 23 187 0 0 23 187 0 0 443 0 0 443 0 222 12	0 72 0 0 0 108 24 60 229 0 12 0 313 12 0 0 12 0 0 12	0 15 392 0 0 45 15 30 0 15 0 15 0 317 0 0 0 0 0 0 0 0 0 0 0 0 0	1 1446 10 16 5 46 12 16 143 5 9 0 0 331 0 0 0 204 8 8 6	0 10 432 0 1137 0 513 30 0 513 30 0 513 30 0 0 201 0 0 201 0 0 20 40 282	0 250 0 0 195 37 371 0 9 278 121 9 9 0 9 9 46 0	0 766 0 523 75 131 0 149 0 0 0 0 377 0 0 0 75 187 0	888 13 1912 0 0 0 0 1111 706 10 209 4 4 4 0 619 6 0 0 0 9 4 0 0	0 248 8 0 8 142 0 8 8 1262 0 8 8 0 0 8 0 0 1216 0 0 0 1216 0 0 0 1216 0 0 1216 0 0 1216 1216	1 337 4 2 11 61 187 4 170 14 8 0 293 3 3 4 70 331 15	0 1387 0 18 0 274 0 18 164 0 402 0 402 0 183 0 0 0 0 9 1 18	0 0 736 14 0 0 55 0 14 95 0 14 95 0 14 0 872 0 872 0 245 14 27 0	0 35 992 0 0 86 43 9 177 233 0 177 0 923 0 9 2 3 9 9 9 9 9 9 9	0 0 119 13 0 46 110 538 2 631 5 2 0 381 5 0 381 5 0 28 259 2	0 0 234 8 0 12 105 2 2 485 1 5 0 129 0 129 0 0 2 1 1 4	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000243565 ENSG0000024381 ENSG0000024381 ENSG0000025759 ENSG0000025759 ENSG0000025759 ENSG00000243319 NCRNA00188 ENSG000002433167 ENSG0000023645 ENSG00000253167 ENSG00000234186
В	0 6 223 10 2 11 174 26 5 223 3 2 2 3 2 2 0 158 1 4 32 3 5 7 7 8 7 7 8 7 7 8 7 7 8 7 8 7 8 7 8 7	0 8 337 27 1 37 131 11 2 693 4 3 1 293 4 0 26 196	0 6 20 3 5 0 245 138 12 857 404 8 0 637 1 0 637 1 0 18 56	0 820 992 516 0 0 133 66 0 145 185 0 0 145 185 0 0 132 291 0 0 0 0 0	0 875 23 0 0 175 0 23 187 0 23 187 0 0 443 0 0 443 0 0 445 222	0 72 0 0 108 24 60 229 0 12 0 313 12 0 313 12 0 0 12	0 15 392 0 0 45 15 30 0 15 0 15 0 317 0 0 0 0 0 0 0 0 0 0 0 0 0	1 1446 10 16 5 46 12 16 143 5 9 0 0 331 0 0 0 331 0 0 0 204 8	0 10 161 0 432 0 11137 0 513 30 0 513 30 0 201 0 0 201 0 0 20 40	0 0 250 0 0 74 195 37 371 0 9 278 121 9 0 9 46	0 766 0 523 75 131 0 149 0 0 0 0 37 0 0 75 187	888 13 1912 0 0 0 1111 706 10 209 4 4 4 0 619 6 19 6 0 0 9 4	0 0 248 8 0 142 0 8 1262 0 8 0 1216 0 0 0 0 1216 0 0 1216 0 0 124 125 125 125 125 125 125 125 125	1 337 4 2 11 61 187 4 170 14 8 0 293 3 0 293 3 4 70 331	0 0 1387 0 18 0 274 0 18 164 0 402 0 402 0 183 0 0 0 0 9 1	0 0 736 14 0 0 55 0 14 95 0 14 95 0 14 0 872 0 872 0 245 14 27	0 35 992 0 86 43 9 17 233 0 177 0 177 0 923 0 0 92 9 9 9 9	0 0 119 13 0 46 110 538 2 631 5 2 631 5 2 0 381 5 0 28 259	0 0 234 8 0 12 105 2 2 2 485 1 5 0 129 0 0 0 2 2 1	ENSG00000230658 ENSG00000235621 <i>NEAT1</i> ENSG00000243565 ENSG00000243565 ENSG00000249381 ENSG00000249381 ENSG00000230513 <i>H19</i> ENSG0000025759 ENSG0000025759 ENSG00000224281 ENSG0000024319 NCRNA00188 ENSG0000023167 ENSG0000023167 ENSG00000237883 <i>MEG3</i>

Figure 4. Expression patterns of IncRNAs in normal human tissues. (A) LncRNAs with the highest overall expression (B) LncRNAs with the highest variance by a coefficient of variation (CV) test. Heatmaps indicate the relative intensity (normalized TPM) of each IncRNA across seventeen human tissues, white blood cells and human embryonic stem cells. Where more than one SAGE library was available, the TPM values were averaged. For the heatmap, the maximum threshold was set at 300 TPM. LncRNAs without names are labeled with an Ensembl ID. doi:10.1371/journal.pone.0025915.g004

expressed 3 (*MEG3*) [48], displayed the highest expression in brain in our dataset (TPM = 677), but showed low level expression in other tissue types (Figure 4). Collectively, these data suggest some lncRNAs may function in a tissue-specific manner. Only $\sim 1\%$ of the lncRNAs were ubiquitously expressed across all tissues examined. These constantly expressed lncRNAs are reminiscent of the expression patterns of "housekeeping" proteincoding genes [49]. The eleven lncRNAs in Table 1 were expressed

Α	Bone Marrow	Brain	Breast	Cartilage	Colon	Embryonic Stem Cells	Esophagus	Gall Bladder	Kidney	Liver	Lung	Lymph Node	Muscle	Ovary	Pelvis	Prostate	Retina	Stomach	Testis	Thyroid	
	780 16 1837 2324 666 1593 731 98 130 114 98 98	546 16 344 630 199 577 321 165 131 89 40 202	543 134 383 613 514 229 131 175 161 127 80 103	3644 1289 277 0 53 11 0 160 11 139 75 53	3321 90 664 144 287 180 90 449 36 503 431 18	88 138 857 705 20 637 282 44 238 84 37 104	106 0 166 197 371 136 93 599 22 193 781 78	288 17 441 518 554 207 352 137 214 186 10 255	30 3460 289 598 80 219 279 40 90 150 0 0	0 133 0 17 0 17 0 663 0 0 0 580 50	668 44 215 639 717 424 160 278 179 100 26 111	83 14 514 417 236 778 292 42 319 125 14 222	375 490 506 571 228 506 326 310 408 685 114 147	187 2896 579 318 56 149 112 149 37 19 112 75	144 239 1017 12 24 48 12 12 12 0 24 0 24 0 24	646 0 1283 633 466 1375 160 38 123 84 23 92	226 0 260 542 34 689 1141 23 271 23 34 475	2124 23 586 505 659 558 269 112 219 20 40 71	121 1884 437 196 151 241 136 121 15 166 0 90	1170 15 201 778 4007 1423 397 110 339 33 19 176	MALAT1 H19 ENSG00000225759 GAS5 NCRNA00188 NEAT1 SNHG6 SCAND2 SNHG5 ENSG00000246638 ENSG00000248360 ENSG00000225733
	130 163 98 0 0 16 0 65	65 59 46 227 1 61 32 34	185 93 106 267 12 62 34 96	458 64 0 11 32 53 96 96	0 18 72 0 0 126 233 359	44 318 37 48 0 246 7 50	44 58 114 53 5 43 862 262	176 117 109 181 110 70 9 104	150 80 130 0 50 70 0 90	17 0 99 0 0 0 414 0	159 53 172 86 429 97 24 38	56 458 42 28 0 472 14 208	49 114 82 0 65 98 16 180	131 0 75 149 262 187 56 0	179 0 24 12 0 0 12 24	46 38 38 15 15 68 53 23	45 418 147 350 0 23 0 23	219 63 72 60 31 87 47 33	121 105 678 0 1010 151 0 166	44 44 13 540 0 32 33 5	NCRNA00152 SNHG1 ENSG00000234072 X/ST ENSG00000224761 ENSG00000224812 ENSG00000249348 ENSG00000250682
						S III								0			T	PM			300
В	Bone Marrow	Brain	Breast	Cartilage	Colon	Embryonic Stem Cells	Esophagus	Gall Bladder	Kidney	Liver	Lung	Lymph Node	Muscle	Ovary	Pelvis	Prostate	Retina	Stomach	Testis	Thyroid	
В	16	16	134	1289	90	138	0	17	3460	Liver	44	14	490	2896	239	0	0	23	1884	15	H19
В	16 666	16 199	134 514	1289 53	90 287	138 20	0 371	17 554	3460 80	133 0	44 717	14 236	490 228	2896 56	239 24	0 466	0 34	23 659	1884 151	15 4007	NEAT1
В	16	16	134	1289	90	138	0	17	3460	133	44	14	490	2896	239	0	0	23	1884	15	
В	16 666 780 0 0	16 199 546 11 1	134 514 543 17 2	1289 53 3644 11 11	90 287 3321 54 0	138 20 88 4 0	0 371 106 255 0	17 554 288 4 0	3460 80 30 0 0	133 0 0	44 717 668 18 0	14 236 83 0 0	490 228 375 0 0	2896 56 187 37 0	239 24 144 0 0	0 466 646 8 0	0 34 226 11 735	23 659 2124 12 0	1884 151 121 0 0	15 4007 1170	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667
В	16 666 780 0 0	16 199 546 11 1 12	134 514 543 17 2 3	1289 53 3644 11 11 842	90 287 3321 54 0 18	138 20 88 4 0 15	0 371 106 255 0 18	17 554 288 4 0 14	3460 80 30 0 0 10	133 0 0 1277 0 0	44 717 668 18 0 4	14 236 83 0 0 14	490 228 375 0 0 0	2896 56 187 37 0 0	239 24 144 0 0 48	0 466 646 8 0 0	0 34 226 11 735 0	23 659 2124 12 0 3	1884 151 121 0 0 60	15 4007 1170 15 0 0	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667 ENSG00000248311
В	16 666 780 0 0 0	16 199 546 11 1 12 1	134 514 543 17 2 3 12	1289 53 3644 11 11 842 32	90 287 3321 54 0 18 0	138 20 88 4 0 15 0	0 371 106 255 0 18 5	17 554 288 4 0 14 110	3460 80 30 0 0 10 50	133 0 0 1277 0 0 0	44 717 668 18 0 4 429	14 236 83 0 0 14 0	490 228 375 0 0 0 65	2896 56 187 37 0 0 262	239 24 144 0 0 48 0	0 466 646 8 0 0 15	0 34 226 11 735 0 0	23 659 2124 12 0 3 31	1884 151 121 0 0 60 1010	15 4007 1170 15 0 0 0	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667 ENSG00000248311 ENSG00000224761
В	16 666 780 0 0	16 199 546 11 1 12	134 514 543 17 2 3	1289 53 3644 11 11 842	90 287 3321 54 0 18	138 20 88 4 0 15	0 371 106 255 0 18	17 554 288 4 0 14	3460 80 30 0 0 10	133 0 0 1277 0 0	44 717 668 18 0 4	14 236 83 0 0 14	490 228 375 0 0 0	2896 56 187 37 0 0	239 24 144 0 0 48	0 466 646 8 0 0	0 34 226 11 735 0	23 659 2124 12 0 3	1884 151 121 0 0 60	15 4007 1170 15 0 0	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667 ENSG00000248311
B	16 666 780 0 0 0 2324 1593 0	16 199 546 11 12 1 630 577 32	134 514 543 17 2 3 12 613 229 34	1289 53 3644 11 11 842 32 0 11 96	90 287 3321 54 0 18 0 144 180 233	138 20 88 4 0 15 0 705 637 7	0 371 106 255 0 18 5 197 136 862	17 554 288 4 0 14 110 518 207 9	3460 80 30 0 10 50 598 219 0	133 0 1277 0 0 0 177 177 414	44 717 668 18 0 4 429 639 424 24	14 236 83 0 0 14 0 417 778 14	490 228 375 0 0 0 65 571 506 16	2896 56 187 37 0 0 262 318 149 56	239 24 144 0 0 48 0 12 48 12	0 466 646 8 0 0 15 633 1375 53	0 34 226 11 735 0 0 0 542 689 0	23 659 2124 12 0 3 31 505 558 47	1884 151 121 0 0 60 1010 196 241 0	15 4007 1170 15 0 0 0 778 1423 33	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667 ENSG00000248311 ENSG00000224761 GAS5 NCRNA00188 ENSG00000249348
B	16 666 780 0 0 0 2324 1593 0 146	16 199 546 11 1 12 1 630 577 32 24	134 514 543 17 2 3 12 613 229 34 29	1289 53 3644 11 11 842 32 0 11 96 0	90 287 3321 54 0 18 0 144 180 233 108	138 20 88 4 0 15 0 705 637 7 7 7	0 371 106 255 0 18 5 197 136 862 748	17 554 288 4 0 14 110 518 207 9 22	3460 80 30 0 0 10 50 598 219 0 0	133 0 1277 0 0 0 177 17 414 133	44 717 668 18 0 4 429 639 424 24 24 14	14 236 83 0 0 14 0 417 778 14 0	490 228 375 0 0 0 65 571 506 16 49	2896 56 187 37 0 0 262 318 149 56 0	239 24 144 0 0 48 0 12 48 12 0	0 466 8 0 0 15 633 1375 53 8	0 34 226 11 735 0 0 542 689 0 0	23 659 2124 12 0 3 31 505 558 47 7	1884 151 121 0 0 60 1010 196 241 0 0	15 4007 1170 15 0 0 0 778 1423 33 0	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667 ENSG00000248311 ENSG00000224761 GAS5 NCRNA00188 ENSG00000249348 ENSG00000232092
B	16 666 780 0 0 0 2324 1593 0	16 199 546 11 12 1 630 577 32	134 514 543 17 2 3 12 613 229 34 29 1	1289 53 3644 11 11 842 32 0 11 96	90 287 3321 54 0 18 0 144 180 233 108 0	138 20 88 4 0 15 0 705 637 7	0 371 106 255 0 18 5 197 136 862 748 5	17 554 288 4 0 14 110 518 207 9	3460 80 30 0 10 50 598 219 0	133 0 1277 0 0 0 177 177 414	44 717 668 18 0 4 429 639 424 24	14 236 83 0 0 14 0 417 778 14	490 228 375 0 0 0 65 571 506 16	2896 56 187 37 0 0 262 318 149 56 0 0 0	239 24 144 0 0 48 0 12 48 12	0 466 646 8 0 0 15 633 1375 53	0 34 226 11 735 0 0 0 542 689 0	23 659 2124 12 0 3 31 505 558 47	1884 151 121 0 0 60 1010 196 241 0	15 4007 1170 15 0 0 0 778 1423 33	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667 ENSG00000248311 ENSG00000224761 GAS5 NCRNA00188 ENSG00000249348 ENSG00000232092 ENSG00000256193
B	16 666 780 0 0 0 2324 1593 0 146 0	16 199 546 11 1 12 1 630 577 32 24 4	134 514 543 17 2 3 12 613 229 34 29	1289 53 3644 11 11 842 32 0 11 96 0 96 96	90 287 3321 54 0 18 0 144 180 233 108	138 20 88 4 0 15 0 705 637 7 7 7 4	0 371 106 255 0 18 5 197 136 862 748	17 554 288 4 0 14 110 518 207 9 22 31	3460 80 30 0 10 50 598 219 0 0 0 0	133 0 0 1277 0 0 0 0 17 17 414 133 17	44 717 668 18 0 4 429 639 424 24 24 14 22	14 236 83 0 0 14 0 417 778 14 0 0 0	490 228 375 0 0 0 65 571 506 16 49 0	2896 56 187 37 0 0 262 318 149 56 0	239 24 144 0 0 48 0 12 48 12 0 526	0 466 8 0 0 15 633 1375 53 8 0	0 34 226 11 735 0 0 542 689 0 0 0 0	23 659 2124 12 0 3 31 505 558 47 7 0	1884 151 121 0 60 1010 196 241 0 0 0 0	15 4007 1170 15 0 0 0 778 1423 33 0 4	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667 ENSG00000248311 ENSG00000224761 GAS5 NCRNA00188 ENSG00000249348 ENSG00000232092
B	16 666 780 0 0 2324 1593 0 146 0 98 0 98 0 1837	16 199 546 11 1 12 1 630 5777 32 24 4 40 1 344	134 514 543 17 2 3 12 613 229 34 29 34 29 1 80 5 383	1289 53 3644 11 11 842 32 0 11 96 0 96 75 0 277	90 287 3321 54 0 18 0 144 180 233 108 0 431 0 664	138 20 88 4 0 15 0 705 637 7 7 7 4 37 3 3 857	0 371 106 255 0 18 5 197 136 862 748 5 781 111 166	17 554 288 4 0 14 110 518 207 9 22 31 22 31 10 9 9 441	3460 80 30 0 10 50 598 219 0 0 0 0 0 0 0 0 0 289	133 0 0 1277 0 0 0 177 17 414 133 17 580 448 0	44 717 668 18 0 4 429 639 424 24 14 24 14 2 6 6 6 6 215	14 236 83 0 0 14 0 417 778 14 0 0 14 0 514	490 228 375 0 0 65 571 506 16 49 0 114 0 506	2896 56 187 37 0 262 318 149 56 0 0 149 56 0 112 0 579	239 24 144 0 0 48 0 12 48 12 0 526 0 0 526 0 0 1017	0 466 8 0 0 15 633 1375 53 8 0 23 0 23 0 1283	0 34 226 11 735 0 0 542 689 0 0 0 0 0 34 0 260	23 659 2124 12 0 3 31 505 558 47 7 0 40 3 3 586	1884 151 121 0 60 1010 196 241 0 0 0 0 0 0 0 0 0 0 0 437	15 4007 1170 15 0 0 778 1423 33 0 4 19 4 19 4 201	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667 ENSG00000248311 ENSG00000224761 GAS5 NCRNA00188 ENSG00000249348 ENSG00000249348 ENSG0000026193 ENSG00000248360 ENSG00000248360 ENSG00000248721 ENSG0000025759
B	16 666 780 0 0 2324 1593 0 146 0 98 0 98 0 1837 16	16 199 546 11 1 12 1 630 5777 32 24 4 40 1 344 8	134 514 543 17 2 3 12 613 229 34 29 1 80 5 383 1	1289 53 3644 11 11 842 32 0 11 96 0 96 75 0 277 0	90 287 3321 54 0 18 0 144 180 233 108 0 431 0 664 0	138 20 88 4 0 15 0 705 637 7 7 4 37 3 3 857 0	0 371 106 255 0 18 5 197 136 862 748 5 781 111 166 9	17 554 288 4 0 14 110 518 207 9 22 31 10 9 22 31 10 9 441 0	3460 80 30 0 10 50 598 219 0 0 0 0 0 0 0 0 0 0 289 0	133 0 0 1277 0 0 0 1277 17 414 133 17 580 448 0 0 0	44 717 668 18 0 4 429 639 424 24 14 24 14 2 26 6 215 1	14 236 83 0 0 14 0 417 778 14 0 0 14 0 514 14	490 228 375 0 0 65 571 506 16 49 0 114 0 506 0	2896 56 187 37 0 262 318 149 56 0 0 149 56 0 0 112 0 579 0	239 24 144 0 0 48 0 12 48 12 0 526 0 0 526 0 0 1017 0	0 466 646 8 0 15 633 1375 53 8 0 23 0 23 0 1283 15	0 34 226 11 735 0 0 542 689 0 0 0 0 34 0 260 350	23 659 2124 12 0 3 31 505 558 47 7 0 40 3 3 586 0	1884 151 121 0 60 1010 196 241 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	15 4007 1170 15 0 0 778 1423 33 0 4 19 4 19 4 201 0	NEAT1 MALAT1 ENSG0000237870 ENSG0000225667 ENSG00002248311 ENSG0000224761 GAS5 NCRNA00188 ENSG0000249348 ENSG00002249348 ENSG00000256193 ENSG00000248360 ENSG00000248721 ENSG00000225759 ENSG00000240204
B	16 666 780 0 0 0 2324 1593 0 146 0 98 0 1837 16 0	16 199 546 11 1 12 1 630 5777 32 24 4 40 1 344 8 1	134 514 543 17 2 3 12 613 229 34 29 34 29 1 80 5 383 1 1 1	1289 53 3644 11 11 842 32 0 11 96 0 96 75 0 277 0 277 0 0 0	90 287 3321 54 0 18 0 144 180 233 108 0 431 0 664 0 0 0	138 20 88 4 0 15 0 705 637 7 7 637 7 7 4 37 3 857 0 0 0	0 371 106 255 0 18 5 197 136 862 748 5 748 5 781 111 166 9 45	17 554 288 4 0 14 110 518 207 9 22 31 10 9 22 31 10 9 441 0 4	3460 80 30 0 10 50 598 219 0 0 0 0 0 0 0 0 0 289 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	133 0 0 1277 0 0 0 177 17 414 133 17 580 448 0 0 332	44 717 668 18 0 4 429 639 424 24 14 24 14 26 6 215 1 1 1	14 236 83 0 14 0 417 778 14 0 0 14 0 514 14 0	490 228 375 0 0 65 571 506 16 49 0 114 0 114 0 506 0 0	2896 56 187 37 0 262 318 149 56 0 0 149 56 0 0 112 0 579 0 0 0 0	239 24 144 0 48 0 12 48 12 48 12 0 526 0 0 526 0 0 1017 0 0	0 466 646 8 0 15 633 1375 53 8 0 23 0 23 0 1283 15 0	0 34 226 11 735 0 0 542 689 0 0 542 689 0 0 0 34 0 0 34 0 260 350 0	23 659 2124 12 0 3 31 505 558 47 7 0 40 3 586 0 3	1884 151 121 0 60 1010 196 241 0 0 0 0 0 0 0 437 0 0 0	15 4007 1170 15 0 0 778 1423 33 0 4 19 4 201 0 0 0	NEAT1 MALAT1 ENSG00000237870 ENSG00000225667 ENSG00000248311 ENSG0000024761 GAS5 NCRNA00188 ENSG0000249348 ENSG00000249348 ENSG00000266193 ENSG00000248701 ENSG00000248721 ENSG00000225759 ENSG00000240204 ENSG0000022382
B	16 666 780 0 0 2324 1593 0 146 0 98 0 98 0 1837 16	16 199 546 11 1 12 1 630 5777 32 24 4 40 1 344 8	134 514 543 17 2 3 12 613 229 34 29 1 80 5 383 1	1289 53 3644 11 11 842 32 0 11 96 0 96 75 0 277 0	90 287 3321 54 0 18 0 144 180 233 108 0 431 0 664 0	138 20 88 4 0 15 0 705 637 7 7 4 37 3 3 857 0	0 371 106 255 0 18 5 197 136 862 748 5 781 111 166 9	17 554 288 4 0 14 110 518 207 9 22 31 22 31 10 9 9 441 0	3460 80 30 0 10 50 598 219 0 0 0 0 0 0 0 0 0 0 289 0	133 0 0 1277 0 0 0 1277 17 414 133 17 580 448 0 0 0	44 717 668 18 0 4 429 639 424 24 14 24 14 2 26 6 215 1	14 236 83 0 0 14 0 417 778 14 0 0 14 0 14 0 514 14	490 228 375 0 0 65 571 506 16 49 0 114 0 506 0	2896 56 187 37 0 262 318 149 56 0 0 149 56 0 0 112 0 579 0	239 24 144 0 0 48 0 12 48 12 0 526 0 0 526 0 0 1017 0	0 466 646 8 0 15 633 1375 53 8 0 23 0 23 0 1283 15	0 34 226 11 735 0 0 542 689 0 0 0 0 34 0 260 350	23 659 2124 12 0 3 31 505 558 47 7 0 40 3 3 586 0	1884 151 121 0 60 1010 196 241 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	15 4007 1170 15 0 0 778 1423 33 0 4 19 4 19 4 201 0	NEAT1 MALAT1 ENSG0000237870 ENSG0000225667 ENSG00002248311 ENSG0000224761 GAS5 NCRNA00188 ENSG0000249348 ENSG00002249348 ENSG00000256193 ENSG00000248360 ENSG00000248721 ENSG00000225759 ENSG00000240204
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Figure 5. Expression patterns of IncRNAs in human cancers. (A) LncRNAs with the highest overall expression (B) LncRNAs with the highest variance by a coefficient of variation (CV) test. Heatmaps indicate the relative intensity (normalized TPM) of each IncRNA across seventeen human cancers and human embryonic stem cells. Where more than one SAGE library was available, the TPM values were averaged. For the heatmap, the maximum threshold was set at 300 TPM. LncRNAs without names are labeled with an Ensembl ID. doi:10.1371/journal.pone.0025915.g005

in at least 90% of 272 SAGE libraries in our dataset, implicating that these transcripts may participate in common biological processes. However, the absolute expression level varied for each tissue, sometimes by hundreds of TPM (Figure 4). This suggests certain lncRNAs may be required at different cellular levels in different tissues or under different conditions, much like many

constitutively expressed protein-coding genes [50,51,52]. The concept of lncRNAs functioning as constitutively expressed regulators has been previously proposed. For example, the lncRNA *XIST* is critical for female development due to its functional role in X-chromosome inactivation [47,53]. Concordantly, a number of the most highly and frequently expressed

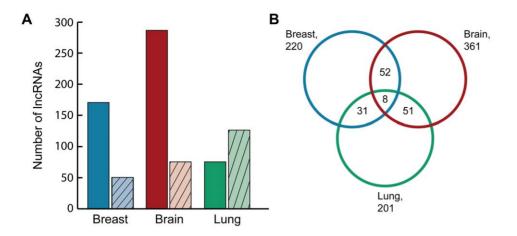


Figure 6. Aberrantly expressed IncRNAs in human cancers. (A) Number of IncRNAs showing significant expression changes. The number of IncRNAs determined to have significant (BH p-value <0.05) differential expression of 2-fold or greater reported. Solid bars indicate upregulated genes, while bars with hatch marks indicate downregulated genes (B) Venn diagram of differentially expressed IncRNAs in human carcinomas. doi:10.1371/journal.pone.0025915.g006

IncRNAs in our dataset have prior associations with key biological processes, including *NEAT1*, a structural scaffold for paraspeckle formation [14,54], *MALAT1* which regulates alternative splicing [31] and small nucleolar RNA host gene 6 (*SNHG6*) which hosts a snoRNA, which function in RNA modification [55]. These findings suggest that lncRNAs may be critical to normal tissue maintenance and function.

In this cross-cancer type analysis, we found that lncRNAs aberrantly expressed in a specific cancer may also be altered in other cancers. For example, while MEG3 is highly expressed in normal brain tissues, this lncRNA was strongly decreased in our brain cancer datasets, and strikingly so in gall bladder, retinal and prostate cancers, consistent with the proposed tumor suppressor role for MEG3 [48,56,57]. In another example, miR155 host gene (miR155HG), a lncRNA processed to the miRNA miR-155, was highly overexpressed in B-cell lymphoma consistent with previous reports [16], but also was also upregulated in esophageal and gall bladder cancers.

Long non-coding RNAs are also implicated in the regulation of embryogenesis [58,59,60]. Fetal lncRNAs reactivated in cancers may represent critical regulators of pluripotency or cellular growth. For example, the lncRNA urothelial cancer associated 1 (UCA1) has demonstrated roles in both embryonic development and is implicated in bladder cancer, supporting this concept [61]. In our datasets, we found several lncRNAs with low expression in normal tissues, but with high expression in both embryonic stem cells and cancer (Table S12). While these reactivated fetal lncRNAs represented mostly uncharacterized examples, H19, a well-studied lncRNA with associations in both mammalian development and cancer [53], was also detected in our dataset. Interestingly, *NEAT1*, which is constitutively and highly expressed in normal tissues [34,62], with the exception of embryonic stem cells, was downregulated in lung, liver, esophageal and retinal cancers (retinoblastoma).

Since genomic amplifications and deletions are key mechanisms of gene deregulation in cancer, we investigated changes in lncRNA expression in genomic regions frequently altered in breast, brain and lung cancer. Comparison of the significantly (p<0.05) deregulated lncRNAs common between brain, breast and lung cancer tissues revealed eight lncRNAs were differentially regulated (\geq 2-fold) compared to normal tissue. Intriguingly, three of these lncRNAs - ENSG00000226380, ENSG0000230937 and ENSG0000253288 - were located on 7q32.3, 1q32.2, and 8q24.23, respectively, in regions completely devoid of protein-coding genes. Like protein-coding genes and miRNAs, it is possible that differential lncRNA expression is driven by similar mechanisms of disruption, including copy number gain/loss or aberrant methylation patterns. Indeed, high level amplification of lncRNA containing loci such as cytoband 19p12



IncRNA						Fold C	hange		Corrected	d p-value				
	Ensembl Gene ID	Chr	Start	End	Strand	Brain	Breast	Lung	Brain	Breast	•			
AC058791.1	ENSG00000230937	7	130565751	130598069	-1	7.00	-3.00	3.59	0.00122	0.02373	0.00000			
CTA-55I10.1	ENSG00000255717	1	209602165	209606183	1	3.37	-2.05	2.72	0.00041	0.00190	0.00000			
NCRNA00263	ENSG00000247556	10	102133372	102143125	1	12.37	2.10	2.46	0.00004	0.00056	0.00000			
AC080037.2	ENSG00000245411	17	70594180	70636611	-1	3.08	2.76	-2.14	0.00009	0.00027	0.00141			
AC012652.1	ENSG00000226380	15	41576203	41601901	1	6.45	3.53	-2.33	0.00026	0.00018	0.03657			
RP11-18C24.6	ENSG00000253288	12	120928131	120933743	-1	-2.48	-3.13	-2.86	0.00405	0.00639	0.01311			
RP11-238K6.1	ENSG00000235823	8	138821687	139095813	-1	7.07	4.18	-4.35	0.00529	0.00012	0.00037			
SNHG1	ENSG00000248008	11	62619460	62623386	-1	3.04	3.27	-5.03	0.00403	0.00043	0.00003			

doi:10.1371/journal.pone.0025915.t002

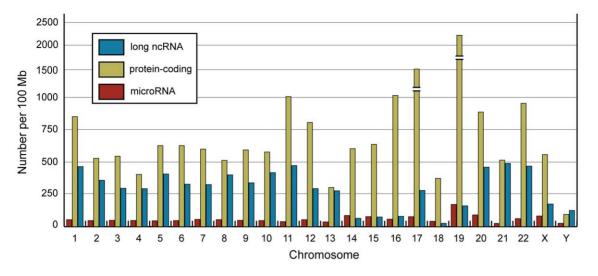


Figure 7. Chromosomal distribution of protein-coding genes, microRNAs and long non-coding RNAs in the human genome. Protein-coding gene (n = 20,655), microRNA (n = 1,746) and long non-coding RNA (n = 9,891) coordinates were downloaded from Ensembl v62 using BioMart. doi:10.1371/journal.pone.0025915.g007

has been reported in breast cancer [63], while high level amplification of 12p13.2 (which contains a number of lncRNA loci) has been reported in breast cancer, glioblastoma, astrocytoma, and squamous cell lung cancer [64,65,66,67]. Likewise, aberrant expression of a number of lncRNAs has been tied to altered methylation patterns [68,69]. However, the mechanism(s) driving aberrant lncRNA expression remains mostly unknown.

While lncRNAs have been documented for nearly three decades, the magnitude and diversity of lncRNA expression has only recently been appreciated. It is estimated that lncRNAs in the human genome number into the tens of thousands, effectively doubling the number of potential gene targets in cancer gene expression networks. Large scale, cross-tissue and cancer studies are crucial to understanding the regulation of lncRNA expression and how these novel transcripts integrate with our current understanding of the mammalian transcriptome. Moreover, a deeper understanding of lncRNA expression will not only expand the number of potential target cancer genes, but also facilitate development of novel anti-cancer therapies, such as gene regulation mediated by antisense RNAs [70] or targeting lncRNA-protein interactions [28].

Materials and Methods

SAGE Libraries

This study uses publically available SAGE libraries for data analysis. A total of 360 SAGE libraries, including 324 from the Cancer Genome Anatomy Project (CGAP) SAGE library collection (GSE15309), 19 lung bronchial epithelium libraries (GSE3707), 13 lung cancer libraries (GSE7898) and 4 never smoker bronchial epithelium libraries (GSE5473), were downloaded from GEO (Table S1). Libraries constructed from nonhuman samples, as well as long SAGE and SAGE-seq libraries were not used in this study. To facilitate direct comparison the SAGE libraries were filtered to retain only those libraries with >50,000 raw tag counts resulting in 272 libraries suitable for analysis (Table S2).

Long non-coding RNA reference list

The lncRNA discovery pipeline is based on a reference list of human lncRNAs curated by the online genomic database Ensembl release 62, built on the Genome Reference Consortium release GRCh37 [71]. The lncRNA reference list was compiled from 1,239 Ensembl (v62) IDs designated as 'lincRNAs' (long intergenic non-coding RNAs, a subclass of lncRNAs) and 8,652 Ensembl IDs (v62) designated as 'processed transcripts' for a total of 9,891 lncRNAs (Table S4). All the lncRNAs used to query the SAGE libraries were Ensembl curated transcripts without a predicted open reading frame. The sequences of all lncRNA transcripts were retrieved from Ensembl (v62) using the Biomart data management system.

SAGE tag-to-gene mapping

Custom Perl scripts were used to create an expression matrix of the unique SAGE tags across the 272 libraries (Perl scripts: getuniquetags.pl and makeTable_April20.pl). The SAGE tags were mapped to Unigene IDs using custom Perl scripts and a short SAGE mapping file (mapping file: Hs_short) downloaded from SAGE Genie (http://cgap.nci.nih.gov/SAGE), to create a matrix of Unigene ID mapped tags and a matrix of unmapped tags (Perl script: extractUnmappedTags_Unigene). The two expression matrices of unmapped tags and Unigene mapped tags were independently filtered to retain only tags with raw tag counts of 2 or more, appearing in at least 3 SAGE libraries.

For the Unigene mapped tags, gene identifiers were assigned to Unigene IDs using SAGE Genie. From this dataset, tags matching known or candidate lncRNAs were extracted manually. Candidate lncRNAs are Unigene IDs with no gene name or matching one or more of the following descriptors: 'non-coding', 'non-protein', 'cDNA', 'transcribed locus', 'clone IMAGE', 'chr(#)orf(#)', 'hypothetical', 'family with sequence similarity', 'FLJ(#)', or 'KIAA(#)'. The candidate lncRNA tags were merged with the unmapped tags and used as a single dataset from which to identify sequence matches to the lncRNA reference list.

The tag-to-gene mapping program SeqMap was used to identify perfect (0 mismatches) tag matches to the transcript sequences from the reference lncRNA list. Tags mapping to lncRNAs were filtered to retain those corresponding to the forward ('sense') strand, while reverse tag matches do not corroborate the expression of the candidate lncRNAs and were not analyzed further. The forward strand tags that mapped to lncRNAs were then combined with the Unigene tags that mapped to lncRNAs to create an expression matrix of SAGE tags mapping to lncRNAs. This matrix was remapped to the lncRNA reference list to confirm accurate tag-to-lncRNA matches.

Data pre-processing

In cases where multiple tags mapped to the same lncRNA, the tags were compressed by summing the tag counts to capture all lncRNA transcript variants and isoforms (Perl script: sumRows.pl). SAGE tags mapping to more than one lncRNA were discarded. Raw tag counts for each SAGE library were normalized to TPM to facilitate adequate comparison among libraries. Additional expression matrices included only SAGE libraries of interest for a given analysis, while removing any columns with unwanted SAGE libraries. These submatrices were filtered to remove lncRNAs with undetected expression. When a tissue or cancer was represented by more than one SAGE library, the normalized TPM were averaged. Finally, all Ensembl v62 IDs were lifted to Ensembl v63, any missing or reassigned IDs were removed from the final lncRNA list.

Statistical analysis

To ensure statistical significance when comparing normal tissues with cancerous tissues, the lncRNA expression matrix was filtered to retain only those tissues represented by a minimum of 5 normal and 5 cancer SAGE libraries. These SAGE libraries were used to derive cancer specific expression matrices. To compare the expression of lncRNAs between normal libraries and cancer libraries, we performed a normalization of expression by permutation of SAGE (NEPS) test as described [41]. LncRNAs with permutation scores of >0.05 were considered to be statistically significant. All fold changes were calculated by dividing the average expression of the cancer SAGE libraries by the average expression of the normal SAGE libraries. Variance calculations were performed by calculating the coefficient of variation (CV) across the averaged normal or cancer SAGE libraries. The lncRNA distribution plots were created by normalizing the number of lncRNAs, miRNAs, or protein-coding genes to 100 megabase (MB) of chromosome and then performing a Spearman correlation.

Supporting Information

Figure S1 Tissue expression profiles of *MALAT1*. Expression was derived from the Human BodyMap 2.0 RNASeq track in Ensembl v62. (JPG)

Figure S2 Tissue expression profiles of NCRNA00188. Expression was derived from the Human BodyMap 2.0 RNASeq track in Ensembl v62.

(JPG)

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Figure S3 Correlation of chromosome distribution between protein-coding genes, miRNAs and lncRNAs. (A) Protein-coding genes compared to miRNAs, (B) Protein-coding genes compared to lncRNAs, (C) lncRNAs compared to miRNAs. The chromosome locations of protein-coding genes (n = 20,655), microRNAs (n = 1746) and long non-coding RNAs (n = 9,891) were downloaded from Ensembl v62. The graphs were generated using GraphPad Prism.

(JPG)

Table S1GEO Libraries.(XLSX)

Table S2Filtered SAGE libraries.(XLSX)

Table S3SAGE library information.(DOC)

Table S4LncRNA reference list.(XLSX)

Table S5LncRNA expression matrix.(XLSX)

Table S6Tag-to-lncRNA matches.(XLSX)

Table S7Normal tissue lncRNA expression matrix.(XLSX)

Table S8Expression validation by BodyMap RNASeq.(XLSX)

 Table S9
 Cancer tissue lncRNA expression matrix.

 (XLSX)
 (XLSX)

Table S10Brain, breast and lung libraries.(XLS)

Table S11 Top differentially expressed brain, breast and lunglncRNAs.

(XLSX)

Table S12ESC and cancers.(XLSX)

Author Contributions

Conceived and designed the experiments: EAG EAV KML CEM SL CJB WLL. Performed the experiments: EAG EAV KSSE GLS JYK. Analyzed the data: EAG EAV KSSE GLS JYK KML DDBS. Contributed reagents/ materials/analysis tools: EAG EAV KSSE GLS JYK KML. Wrote the paper: EAG EAV KSSE CEM SL CJB WLL.

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