The Transcribed-Ultraconserved Regions: A Novel Class of Long Noncoding RNAs Involved in Cancer Susceptibility

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During recent years, novel approaches and new technologies have revealed a startling level of complexity of higher eukaryotes' transcriptome. A large proportion of the transcriptional output is represented by protein noncoding RNAs (ncRNAs) that arise from the "dark matter" of the genome. Focus on such sequences has revealed numerous RNA subtypes with several functions in RNA processing and gene expression regulation, and deep sequencing studies imply that many remain to be discovered. This review gives a picture of the state of the art of a novel class of long ncRNA known as transcribed-ultraconserved regions (T-UCRs). Most recent studies show that they are significantly altered in adult chronic lymphocytic leukemias, carcinomas, and pediatric neuroblastomas, leading to the hypothesis that UCRs may play a role in tumorigenesis and promising innovative future T-UCR-based therapeutic approaches.

KEYWORDS: ultraconserved regions, noncoding RNA, gene expression

INTRODUCTION

Since their introduction in the mid-1990s, microarrays have rapidly become a high-throughput method of gene expression analysis in relation to physiology, development, and disease. Moreover, together with sequencing of the human genome as well as those of model organisms, they largely contributed to the exploration of the complexities of eukaryotic genomes[1]. In the last few years, there has been increasing evidence that ~98% of human DNA is transcribed into molecules that are protein noncoding RNAs (ncRNAs)[2,3]. Such a startling finding has revolutionized the central dogma of molecular biology, according to information flows from DNA to protein through RNA as its intermediary[4]. From there, it was easy to generalize that "one gene equals one protein, one function". Generally, this holds true in prokaryotes, whose genomes consist of tightly packed protein-coding sequences, whereas complex eukaryotes have absolutely different patterns of functional regulation. Thus, the modern view of the eukaryotic RNA world involves many ncRNAs, which process and regulate other RNA molecules by cleavage, nucleotide modification, transcription, and degradation[5]. Numerous subtypes of ncRNAs participate in such RNA settings, including rRNAs, mRNAs, tRNAs, mitochondrial ncRNAs[6], small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), several classes of regulatory RNAs involved

in RNA interference (RNAi), and many long intergenic ncRNAs (lincRNAs)[7,8,9], such as *HOTAIR*[10]. In the last years, significant progress has been made on functional annotation of a particular class of small ncRNAs, namely microRNAs (miRNAs). These are short RNA molecules, on average only 22 nucleotides long, that both work as negative post-transcriptional regulators of gene expression through imperfect binding to target mRNAs[11] and also induce up-regulation of target mRNAs, i.e., during the cell cycle[12]. Conversely, the functionality of long ncRNAs is still elusive. Some long ncRNAs are located and transcribed within the intergenic stretches and they associate with chromatin-modifying complexes to alter gene expression[13,14]. The majority of long ncRNAs are transcribed as complex networks of overlapping sense and antisense transcripts that often include protein-coding genes[15]. Genomic sequences of these transcriptional foci are often shared within a number of different coding and noncoding transcripts in the sense and antisense directions, giving rise to a complex hierarchy of overlapping isoforms[16].

Many small RNAs, such as miRNAs or snoRNAs, and a representative set of human long ncRNAs, exhibit strong conservation at the levels of sequence, promoter, and splicing across diverse species[17,18], which is indicative of positive selection[19].

THE ULTRA-CONSERVED REGIONS

Ultraconserved regions (UCRs) were discovered in 2004 by bioinformatics comparisons of the mouse, rat, and human genomes[20]. They are 481 genomic elements longer than 200 bp (range: 200–779 bp) that are absolutely conserved (100% identity with no insertions or deletions) among the three species. Even single-nucleotide polymorphisms (SNPs) are under-represented within UCRs[20,21] and ultraconserved elements do not accumulate mutations in somatic cells in conditions of genomic instability[22]. Such extreme conservation could be a sign of a strong purifying selection[23]. As a proof of principle of the high levels of conservation of UCRs during evolution, a distal enhancer and an ultraconserved exon, originating from the short interspersed repetitive element retroposon family active in lobe-finned fishes and terrestrial vertebrates more than 400 million years ago, have been found still active in a “living fossil” Indonesian coelacanth[24].

Originally, focusing on overlap with protein-coding regions based on hg17 genome assembly, 111 (23%) UCRs were categorized as exonic and 256 (53%) as nonexonic, whereas the remaining 114 (24%) UCRs, for which the evidence for transcription was inconclusive, were called possibly exonic[20]. Recently, Mestdagh and colleagues[25] reannotated all UCR sequences using the more recent genome build hg18 and reorganized them into five different categories (intergenic, intronic, exonic, partly exonic, exon containing) by matching their location to that of the human RefSeq genes. The new genomic categories are unambiguously defined and provide a more detailed genomic annotation for each UCR: 38.7% UCRs, located between genes, are termed as intergenic; 42.6% as intronic; 4.2% as exonic; 5% as partly exonic; and 5.6% as exon containing. For a few UCRs (3.9%), the genomic annotation varies because of host gene splice variants and these UCRs are categorized as “multiple”.

Intergenic UCRs are often found in “gene deserts” that extend more than 1 Mb. Precisely, 140 nonexonic elements are more than 10 kb away from any known gene, and 88 are more than 100 kb away[20]. The set of 156 annotated genes that flank intergenic UCRs is significantly enriched for genes involved in early developmental tasks, suggesting that many of the associated UCRs may be distal enhancers of these developmental genes[26]. For instance, one of these elements (uc.351) is contained in an enhancer situated about 225 kb upstream of *DACH* (homolog of the *Drosophila* dachshund gene), known to be involved in the development of brain, limbs, and sensory organs[20,27]. Also, intronic UCRs are often associated with developmental genes. These include the neuroretina-specific enhancer in the fourth intron of *PAX6* (uc.328)[20,28].

There are 93 known protein-coding genes that overlap with exonic UCRs. Such genes show significant functional enrichment for RNA binding (i.e., *HNRPK*, *HNRPH1*, *HNRPU*, *HNRPD*, *HNRPM*, *SFRS1*, *SFRS3*, *SFRS6*, *SFRS7*, *SFRS10*, *SFRS11*, *TRA2A*, *PCBP2*, and *PTBP2*), regulation of

splicing, RNA recognition motifs (including the six members of the *SFRS* family), as well as DNA binding motifs, in particular the Homeobox domain. These attributes are enriched in the 225 protein-coding genes that are near the nonexonic UCRs as well, although less significantly, suggesting that exonic UCRs may be specifically associated with RNA processing and nonexonic elements with regulation of transcription at DNA level[20].

The UCRs are frequently located at fragile sites and cancer-associated genomic regions (CAGRs), such as minimal regions of amplification and of loss of heterozygosity[29] (Supplementary Table 1). Intriguingly, those UCRs differentially expressed in human cancers are located in CAGRs specifically associated with that type of cancer. This is the case of uc.349A and uc.352 differentially expressed between normal and leukemic CD5-positive cells[30]: they are located within the 13q21.33–q22.2 chromosomal region, which has been linked to susceptibility to familial chronic lymphocytic leukemia[31]. Also, in pediatric neuroblastoma (NB) tumors, expression of seven UCRs correlated to their copy-number status[25]. Together, these data suggest that not only the protein-coding genes, but also the UCRs located in the CAGRs, could be candidate players for cancer susceptibility.

TRANSCRIBED-UCRs

A large fraction of UCRs are transcribed (so-called transcribed-UCRs, T-UCRs) in normal human tissues, and their expression levels show both a ubiquitous (for 34% of T-UCRs) and a tissue-specific pattern[30]. The T-UCRs show predominant transcription from one strand and only 9% of them are bidirectionally transcribed[30].

Interestingly, in addition to microarrays, Northern blot, and reverse transcription-quantitative real-time PCR (RT-qPCR)[29], a linear isothermal Ribo-SPIA™ RNA amplification method enables sensitive and accurate high-throughput interrogation of all 481 T-UCRs[32]. This is particularly important since transcription regulation studies are increasingly conducted in small samples of potential clinical interest, such as tumor biopsies, laser capture microdissected or sorted cell populations, when limited starting RNA amounts are available.

T-UCR AND TUMORS

Calin et al.[30] were the first to investigate the expression of UCRs in human cancers, focusing on chronic lymphocytic leukemia, the most frequent adult leukemia in the Western world[33], on colorectal carcinoma, one of the most common cancers in industrialized countries[34], and on hepatocellular carcinoma, the most rapidly increasing type of cancer in the U.S.[35]. They found that, for all the tumor types examined, the malignant cells have a unique spectrum of expressed UCRs when compared with the corresponding normal cells, suggesting that variations in T-UCR expression are involved in the malignant process. Moreover, distinct T-UCR expression signatures were differentially expressed in leukemias and carcinomas, and thus they might offer a novel strategy for cancer diagnosis and prognosis.

Recently, we investigated T-UCR expression in NB tumors[36]. The NB is a pediatric tumor of the sympathetic nervous system characterized by a remarkable heterogeneous clinical behavior[37]. Patients with localized NB have a favorable outcome and in infants with disseminated stage-4 tumor, the progression of disease is generally halted by good response to therapy. Conversely, only 20–30% of children older than 12–18 months of age with a stage-4 tumor show progression free– and overall survival longer than 60 months, despite multimodal therapeutic protocols[38]. In recent years, several prognostic signatures derived from gene expression profiles, DNA abnormalities, and miRNAs have been proposed as sensitive indicators of tumor progression in NB patients[39,40,41,42,43]. Although efforts have been performed in order to validate each gene classifier on independent patient cohorts[44,45], the major challenges remain to identify additional tumor-specific prognostic markers for improved risk estimation at the time of diagnosis, especially in high-risk NB patients. For the first time, we defined a signature based

on 28 T-UCRs that is associated with good outcome in noninfant patients diagnosed with metastatic NB[36]. More recently, Mestdagh et al.[25] confirmed that T-UCRs are widely expressed in NB tumors and correlate to clinical-genetic parameters, such as *MYCN* oncogene status.

As regarding dysregulation of T-UCR transcription in cancer, Calin et al.[30] demonstrated that transcription of tumor-associated T-UCRs in leukemias is negatively regulated by direct interaction with miRNAs. Similarly, we found negative correlations between expression values of nine specific T-UCRs and five predicted interactor miRNAs of the signature able to differentiate between long- and short-surviving high-risk NB patients[36]. In both studies, sequence complementarity gives rise to several miRNA:UCR interacting pairs, indicating complex redundancy in regulatory mechanisms between miRNAs and T-UCRs. Accordingly, these findings provide support for a model in which both coding and noncoding genes are involved in and cooperate in human tumorigenesis. Notably, it is now easily possible to match miRNA and UCR sequences by a specific database, namely Ucbase & miRfunc, which provides UCR sequence data and shows miRNA function[46].

To gain further insight into the initiation and regulation of T-UCR transcription, Mestdagh et al.[25] evaluated the chromatin state of the T-UCR genomic neighborhood. Both inter- and intragenic T-UCRs are significantly associated with active trimethylation of lysine 4 of histone H3 (H3K4me3), a marker for transcriptional initiation[47,48], but with a different distribution as compared with protein-coding genes, suggesting a difference in transcriptional organization between T-UCRs and protein-coding genes. In addition, H3K4me3 distance distributions for miRNAs and T-UCRs appear similar, suggesting common features of transcription organization for these two classes of ncRNA, with initiation sites several kilobases away[49].

Finally, epigenetic mechanisms as potential regulators of T-UCR expression have been evaluated. We found that 78% of intragenic T-UCRs deregulated in high-risk NBs are associated with CpG islands in the promoter region of their own host genes[36]. Therefore, much like CpG island hypermethylation-mediated silencing of miRNAs with tumor-suppressor features contributes to human cancer[50], the global DNA hypermethylation events in unfavorable NB[51,52] may also affect T-UCR-host genes, and thus silence T-UCRs with a potential oncogenic role. Recently, a pharmacological and genomic approach confirmed the possible existence of an aberrant epigenetic pattern of T-UCRs. Indeed, Lujambio and coauthors[53] observed that while almost half of the UCR-associated CpG islands are unmethylated in all tissues, the other half show tissue-specific UCR CpG island methylation, as occurs with promoter CpG islands of coding genes[54] and miRNAs[55]. Moreover, treatment of cancer cells with the DNA methylation inhibitor 5-aza-2-deoxycytidine disclosed that epigenetic inactivation by CpG island hypermethylation of a subset of T-UCRs occurs in a wide spectrum of human cancer cell lines and primary tumors. Taken together, these findings support a model in which epigenetic disruption of T-UCRs constitutes a hallmark of human tumorigenesis. Accordingly, tumor-specific CpG island hypermethylated UCRs may be useful biomarkers of disease.

Table 1 summarizes the main studies on the field of UCRs and cancer, and the main findings of each of these studies.

FUNCTION OF THE ULTRA-CONSERVED ELEMENTS

The remarkably high degree of conservation across species implies that UCRs may have a fundamental functional importance for the ontogeny and phylogeny of mammals and other vertebrates. Although UCRs are significantly depleted among segmental duplications and copy-number variants[56], deletion of some of these regions in knock-out mice was not associated to any notable phenotype abnormality[57]. Therefore, the role of UCRs in viability is still controversial.

TABLE 1
List of Studies on the Field of UCRs and Cancer and Main Findings of Each

Ref.	Tumor Type	Main Findings
Calin et al.[30]	Chronic lymphocytic leukemia	Identification of distinct tumor-associated UCR expression signatures.
	Colorectal carcinoma	UCRs are frequently located at fragile sites and genomic regions involved in cancers.
	Hepatocellular carcinoma	Expression of certain UCRs may be regulated by miRNAs abnormally expressed in human chronic lymphocytic leukemia. Inhibition of overexpressed uc.73A induces apoptosis in colon cancer cells.
Scaruffi et al.[36]	Neuroblastoma	Identification of a signature based on T-UCR expression that is associated with good outcome in noninfant patients diagnosed with metastatic neuroblastoma.
Mestdagh et al.[25]	Neuroblastoma	Correlations between specific T-UCR expression levels and clinicogenetic parameters. Assignments of T-UCRs to cellular processes such as <i>TP53</i> response, differentiation and proliferation.
Lujambio et al.[53]	Colon cancer Breast cancer Lung cancer Melanoma Leukemia Lymphoma	Hypermethylation of T-UCR CpG islands.

Based on the evidence of most recent studies, UCRs are believed to be important in vertebrate genomes, i.e., for long-range enhancer-like activity[58,59,60] (Supplementary Table 2), for homeostatic maintenance of splicing factor expression levels involved in post-transcriptionally gene regulation by alternative splicing coupled with nonsense-mediated mRNA decay[61,62], for regulation of transcription, both as epigenetic modification marks[63,64], and as transcriptional coactivators[65].

In cancer cells, T-UCRs might act as oncogenes. Indeed, functional analysis involving small interfering RNAs have identified one T-UCR in colorectal cancer, namely uc.73A, to be oncogenic by increasing the number of malignant cells as a consequence of reduced apoptosis[30]. To go into more depth of the processes in which T-UCRs are involved, Mestdagh and colleagues[25] implemented an integrative genomic workflow to infer putative T-UCR functions using Gene Set Enrichment Analysis[66] and validated them using *in vitro* systems. For a large number of T-UCRs, they observed widespread association to numerous cancer-related cellular functions and pathways, such as proliferation, apoptosis, and differentiation. For example, the most prominent cluster identified using this methodology contained several T-UCRs significantly related to the expression of protein-coding genes involved in cell cycle, DNA replication, and DNA repair.

CONCLUSION AND FUTURE PERSPECTIVES

It has been well accepted that T-UCRs are regulatory elements within the RNA-processing machinery that also play a critical role in human diseases such as cancer. Indeed, malignant cells show specific alterations not only at genes coding for oncoproteins or tumor suppressors, but also at several classes of ncRNAs. Dysregulation of T-UCRs is a common feature of human cancer. It offers the prospect of defining both tumor-specific signatures of T-UCRs and tumor-specific methylated UCRs that are

associated with diagnosis, prognosis, and response to treatments. Above all, aberrant UCR methylation in the transformed cells might provide a molecular basis for the innovative therapeutic use of DNA-demethylating compounds in the treatment of cancer patients. As a proof of principle, restoration of expression of a down-regulated T-UCR, or, alternatively, inhibition of an overexpressed T-UCR by a small interfering RNA approach could reverse the tumor phenotype. Moreover, localization of such ncRNA within CAGRs could open the way for starting T-UCR-based therapy trials.

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SUPPLEMENTARY TABLE 1
Annotation of T-UCR Located at CAGR According to Genome Build hg18

UCR Name	Location	Start (bp)	End (bp)
uc.1	Chromosome 1	10520284	10520490
uc.2	Chromosome 1	10655130	10655336
uc.3	Chromosome 1	10673752	10673976
uc.4	Chromosome 1	10680836	10681194
uc.5	Chromosome 1	10703938	10704151
uc.6	Chromosome 1	10717708	10718008
uc.7	Chromosome 1	10758720	10758975
uc.8	Chromosome 1	10774405	10774620
uc.9	Chromosome 1	10847997	10848198
uc.10	Chromosome 1	10888161	10888435
uc.21	Chromosome 1	48885573	48885807
uc.22	Chromosome 1	50778819	50779126
uc.23	Chromosome 1	50808365	50808593
uc.24	Chromosome 1	50871733	50872068
uc.25	Chromosome 1	50938622	50938856
uc.26	Chromosome 1	63142234	63142445
uc.27	Chromosome 1	63142468	63142757
uc.28	Chromosome 1	70469301	70469655
uc.35	Chromosome 1	97774870	97775074
uc.38	Chromosome 1	162206579	162206802
uc.39	Chromosome 1	162290159	162290514
uc.40	Chromosome 1	162904586	162904832
uc.62	Chromosome 2	60634186	60634419
uc.63	Chromosome 2	61606005	61606282
uc.66	Chromosome 2	73028511	73028757
uc.95	Chromosome 2	171279776	171280026
uc.96	Chromosome 2	172528920	172529180
uc.97	Chromosome 2	172530877	172531318
uc.98	Chromosome 2	172664764	172665001
uc.99	Chromosome 2	172666627	172667024
uc.108	Chromosome 2	176648603	176648976
uc.109	Chromosome 2	177211584	177211807
uc.110	Chromosome 2	236736121	236736363
uc.111	Chromosome 3	9446461	9446756
uc.116	Chromosome 3	70649114	70649319
uc.117	Chromosome 3	70954530	70954780
uc.118	Chromosome 3	70954782	70955000
uc.135	Chromosome 3	170316977	170317177
uc.137	Chromosome 3	181919551	181919935
uc.139	Chromosome 4	4574341	4574678
uc.140	Chromosome 4	12618968	12619190
uc.141	Chromosome 4	24138260	24138554
uc.145	Chromosome 4	105565762	105566009
uc.146	Chromosome 4	112135925	112136138

uc.150	Chromosome 5	3565621	3565882
uc.151	Chromosome 5	32415894	32416107
uc.152	Chromosome 5	50371395	50371595
uc.173	Chromosome 5	133754059	133754334
uc.177	Chromosome 5	170350234	170350490
uc.178	Chromosome 5	170350603	170350851
uc.179	Chromosome 5	170560817	170561035
uc.180	Chromosome 5	170561094	170561318
uc.181	Chromosome 5	170562084	170562361
uc.182	Chromosome 5	170635684	170635922
uc.183	Chromosome 5	171317125	171317360
uc.193	Chromosome 6	86378405	86378723
uc.194	Chromosome 6	94025785	94025985
uc.203	Chromosome 6	163911694	163911896
uc.210	Chromosome 7	26663603	26663859
uc.211	Chromosome 7	26695997	26696287
uc.212	Chromosome 7	27108463	27108667
uc.213	Chromosome 7	27149657	27149857
uc.220	Chromosome 7	96471852	96472108
uc.221	Chromosome 7	96479237	96479585
uc.222	Chromosome 7	113844407	113844607
uc.223	Chromosome 7	113845421	113845688
uc.224	Chromosome 7	113850255	113850549
uc.225	Chromosome 7	113860091	113860291
uc.226	Chromosome 7	113996554	113996758
uc.227	Chromosome 7	114082552	114082782
uc.228	Chromosome 7	114903933	114904197
uc.229	Chromosome 7	114921881	114922176
uc.230	Chromosome 7	115106697	115106934
uc.231	Chromosome 7	115369353	115369576
uc.235	Chromosome 8	25831838	25832064
uc.243	Chromosome 8	77853517	77853732
uc.246	Chromosome 8	119192399	119192682
uc.265	Chromosome 9	107158292	107158508
uc.266	Chromosome 9	108418113	108418355
uc.267	Chromosome 9	124093711	124093913
uc.268	Chromosome 9	124646649	124646899
uc.269	Chromosome 9	125577758	125577974
uc.270	Chromosome 9	127343890	127344167
uc.271	Chromosome 9	127344173	127344383
uc.272	Chromosome 9	127472409	127472621
uc.273	Chromosome 9	127557419	127557739
uc.274	Chromosome 9	127561692	127562018
uc.275	Chromosome 9	127623937	127624191
uc.276	Chromosome 9	127645633	127646064
uc.277	Chromosome 9	127647531	127647806
uc.278	Chromosome 9	127685986	127686222
uc.279	Chromosome 9	127712380	127712715

uc.280	Chromosome 9	127717827	127718046
uc.282	Chromosome 9	139162311	139162517
uc.293	Chromosome 10	102362659	102362901
uc.294	Chromosome 10	102363607	102364050
uc.295	Chromosome 10	102365090	102365298
uc.296	Chromosome 10	102405096	102405556
uc.297	Chromosome 10	102409210	102409573
uc.298	Chromosome 10	102437648	102438006
uc.299	Chromosome 10	102499425	102499634
uc.300	Chromosome 10	102537108	102537315
uc.301	Chromosome 10	102557781	102558064
uc.302	Chromosome 10	102969170	102969510
uc.303	Chromosome 10	103042417	103042688
uc.304	Chromosome 10	103072494	103072765
uc.305	Chromosome 10	103201425	103201729
uc.306	Chromosome 10	103202029	103202252
uc.307	Chromosome 10	103233973	103234204
uc.308	Chromosome 10	103235802	103236078
uc.309	Chromosome 10	103257021	103257288
uc.310	Chromosome 10	114394213	114394441
uc.311	Chromosome 10	120064392	120064610
uc.312	Chromosome 10	120066527	120066848
uc.317	Chromosome 10	131336328	131336545
uc.318	Chromosome 10	131581576	131581896
uc.330	Chromosome 11	66150472	66150678
uc.333	Chromosome 11	124149857	124150126
uc.338	Chromosome 12	52144756	52144978
uc.339	Chromosome 12	52357363	52357614
uc.340	Chromosome 12	52377099	52377357
uc.341	Chromosome 12	52669185	52669498
uc.342	Chromosome 12	52696761	52696987
uc.343	Chromosome 12	52708708	52709095
uc.344	Chromosome 12	52713153	52713406
uc.345	Chromosome 12	52733867	52734167
uc.347	Chromosome 13	70691989	70692197
uc.348	Chromosome 13	70961358	70961597
uc.349	Chromosome 13	71019303	71019505
uc.350	Chromosome 13	71154101	71154340
uc.351	Chromosome 13	71566901	71567155
uc.352	Chromosome 13	71592166	71592365
uc.353	Chromosome 13	71669554	71669876
uc.355	Chromosome 13	94416883	94417110
uc.356	Chromosome 13	96806821	96807071
uc.357	Chromosome 13	111764338	111764579
uc.361	Chromosome 14	28302886	28303152
uc.362	Chromosome 14	28418503	28418741
uc.363	Chromosome 14	28931070	28931334
uc.364	Chromosome 14	29782510	29782716

uc.365	Chromosome 14	29812113	29812390
uc.366	Chromosome 14	30452458	30452659
uc.367	Chromosome 14	32914214	32914511
uc.368	Chromosome 14	33138327	33138554
uc.369	Chromosome 14	33192368	33192580
uc.370	Chromosome 14	33272322	33272714
uc.371	Chromosome 14	35089940	35090235
uc.372	Chromosome 14	35112786	35113062
uc.373	Chromosome 14	35651558	35651951
uc.374	Chromosome 14	36785664	36785887
uc.375	Chromosome 14	36846971	36847270
uc.379	Chromosome 14	96501121	96501372
uc.380	Chromosome 14	96832347	96832578
uc.381	Chromosome 14	96949043	96949280
uc.382	Chromosome 15	33706204	33706403
uc.383	Chromosome 15	34607219	34607487
uc.384	Chromosome 15	34752685	34752950
uc.385	Chromosome 15	34972962	34973170
uc.386	Chromosome 15	35309301	35309503
uc.413	Chromosome 17	34820045	34820316
uc.415	Chromosome 17	44018905	44019111
uc.416	Chromosome 17	44025886	44026171
uc.417	Chromosome 17	44037311	44037532
uc.418	Chromosome 17	53437227	53437443
uc.419	Chromosome 17	53437714	53438002
uc.444	Chromosome 19	35186619	35187006
uc.445	Chromosome 19	35258275	35258584
uc.446	Chromosome 19	35439694	35439965
uc.447	Chromosome 19	35459621	35459893
uc.458	Chromosome 22	34478438	34478641

SUPPLEMENTARY TABLE 2
List of T-UCR with a Known Enhancer Activity

uc.2	uc.41	uc.94	uc.137	uc.180	uc.222	uc.269	uc.355	uc.421	uc.463
uc.5	uc.47	uc.98	uc.140	uc.181	uc.223	uc.288	uc.358	uc.425	uc.467
uc.8	uc.54	uc.100	uc.145	uc.190	uc.227	uc.300	uc.363	uc.430	uc.470
uc.10	uc.55	uc.104	uc.150	uc.192	uc.236	uc.309	uc.365	uc.435	uc.476
uc.15	uc.60	uc.105	uc.152	uc.196	uc.245	uc.314	uc.371	uc.437	uc.482
uc.18	uc.65	uc.108	uc.157	uc.198	uc.248	uc.315	uc.383	uc.438	
uc.19	uc.67	uc.109	uc.163	uc.204	uc.249	uc.316	uc.388	uc.444	
uc.23	uc.78	uc.110	uc.164	uc.206	uc.250	uc.322	uc.389	uc.445	
uc.25	uc.82	uc.112	uc.165	uc.207	uc.252	uc.325	uc.392	uc.447	
uc.26	uc.85	uc.119	uc.168	uc.211	uc.254	uc.329	uc.396	uc.448	
uc.27	uc.87	uc.123	uc.170	uc.214	uc.260	uc.334	uc.400	uc.449	
uc.29	uc.88	uc.124	uc.175	uc.215	uc.261	uc.335	uc.410	uc.450	
uc.31	uc.92	uc.125	uc.179	uc.220	uc.262	uc.353	uc.411	uc.462