Front. Med. 2019, 13(3): 330–343
https://doi.org/10.1007/s11684-017-0557-0

RESEARCH ARTICLE

Overexpressed long noncoding RNA CRNDE with distinct alternatively spliced isoforms in multiple cancers

Xuefei Ma[1,*], Wei Zhang[1,2,*], Rong Zhang[3], Jingming Li[1], Shufen Li[1], Yunlin Ma[1], Wen Jin (✉)[1], Kankan Wang (✉)[1,2]

[1]*State Key Laboratory of Medical Genomics and Shanghai Institute of Hematology, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China;* [2]*School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China;* [3]*Department of Hematology, Xi'an Gaoxin Hospital, Xi'an 710075, China*

Abstract **Alternative splicing is a tightly regulated process that contributes to cancer development. CRNDE is a long noncoding RNA with alternative splicing and is implicated in the pathogenesis of several cancers. However, whether deregulated expression of CRNDE is common and which isoforms are mainly involved in cancers remain unclear. In this study, we report that CRNDE is aberrantly expressed in the majority of solid and hematopoietic malignancies. The investigation of CRNDE expression in normal samples revealed that CRNDE was expressed in a tissue- and cell-specific manner. Further comparison of CRNDE expression in 2938 patient samples from 15 solid and hematopoietic tumors showed that CRNDE was significantly overexpressed in 11 malignancies, including 3 reported and 8 unreported, and also implicated that the overexpressed isoforms differed in various cancer types. Furthermore, anti-cancer drugs could efficiently repress CRNDE overexpression in cancer cell lines and primary samples, and even had different impacts on the expression of CRNDE isoforms. Finally, experimental profiles of 12 alternatively spliced isoforms demonstrated that the spliced variant CRNDE-g was the most highly expressed isoform in multiple cancer types. Collectively, our results emphasize the cancer-associated feature of CRNDE and its spliced isoforms, and may provide promising targets for cancer diagnosis and therapy.**

Keywords long noncoding RNA; CRNDE; alternative splicing

Introduction

Alternative splicing is an important process in post-transcriptional regulation and significantly increases the complexity of the transcriptome by generating multiple splice isoforms. The process of alternative splicing is regulated in a tissue- and cell-specific manner and contributes to the pathogenesis of many cancers. Consequently, cancer signatures based on specific isoforms are usually more precise than those based on gene expression [1]. Thus, the cancer-specific isoforms may serve as new diagnostic biomarkers to distinguish various cancer types and provide potential targets for drug discovery [2]. Therefore, identifying and characterizing alternative splicing isoforms are essential for generating specific cancer signatures and developing new strategies for precision medicine.

Recent advances in high-throughput sequencing technologies have elucidated that in human genome the number of long noncoding RNAs (lncRNAs) is significantly larger than that of coding genes, and many lncRNAs have multiple alternatively spliced isoforms [3–5]. lncRNAs are recognized as important regulators of gene expression and play key roles in tumor initiation, progression, and metastasis [6,7]. For example, lncRNA DANCR and HOTTIP are closely related to tumor progression and poor prognosis in patients with colorectal cancer (CRC) [8,9]. HOTAIRM1 is a myeloid-specific lncRNA, and its deregulation is involved in leukemogenesis and impacts prognosis in some types of acute myeloid leukemia (AML) [10]. Thus, tissue-specific or cancer-specific lncRNAs emerge as a novel assortment of molecular biomarkers for cancer prognosis.

Alternative splicing of lncRNAs further expands their

Received March 20, 2017; accepted April 30, 2017
Correspondence: Wen Jin, yephero@hotmail.com;
Kankan Wang, kankanwang@shsmu.edu.cn

*These authors contributed equally to this work.

regulatory and functional complexity in tumorigenesis. lncRNAs are predominately transcribed by RNA polymerase II and often multiexonic and polyadenylated. Therefore, the alternative splicing of lncRNAs may be executed through canonical splice sites, which is similar to that of protein-coding mRNAs [11]. Especially, lncRNAs tend to undergo incomplete splicing due to the co-transcriptional splicing weakening close to the polyadenylation site [12]. In addition, studies have shown that lncRNAs can be transcribed by RNA polymerase III (Pol III), which has also been reported to drive alternative splicing through Pol III-transcribed noncoding RNA such as 38A and 17A [13]. Therefore, determination of the differential expression pattern of different lncRNA isoforms in cancer can be of help to more precisely identify cancer-specific biomarkers, provide potential targets for developing new therapeutic strategies, and elucidate the accurate regulation mechanisms of lncRNAs in tumorigenesis.

Colorectal Neoplasia Differentially Expressed (non-protein-coding) (gene symbol *CRNDE*) was first identified to be overexpressed in CRC [14]. Later on, CRNDE has also been found to be upregulated in patients with pancreatic cancer [15] and glioma [16]. It is located on chromosome 16 and adjacent to the *IRX5* gene on the opposite strand. Studies have shown that *CRNDE* and *IRX5* genes share a bidirectional promoter, and their expression can be coregulated in a concordant pattern [17]. CRNDE confers multiple functions in tumorigenesis. It can promote tumor cell proliferation and chemoresistance through Wnt/β-catenin signaling in CRC and renal cell carcinoma [18,19]. CRNDE can also exert migration and invasion effects in CRC cells and glioma stem cells and inhibit cell apoptosis [20]. Notably, it has at least 12 different alternative transcript isoforms generated from six exons at the *CRNDE* locus. Among these isoforms, the CRNDE-h isoform has been reported to be specifically upregulated and associated with poor prognosis of CRC and glioma [21,22]. This finding implicates that the spliced variants of CRNDE, particularly the CRNDE-h isoform, might exhibit tissue-specific cancer types. However, the expression pattern of CRNDE and its various spliced isoforms in the majority of cancer types and drug influence on its expression remain unclear.

In this study, we retrieved the gene expression data sets of 109 normal tissues, 67 normal cell types, and 15 types of solid and hematopoietic tumors including 2938 patient samples to investigate CRNDE distribution in normal tissues and its abnormal expression in cancer samples. We found that CRNDE was expressed in a tissue- and cell-specific pattern. More importantly, we showed that CRNDE was overexpressed in 11 of 15 tested cancer types, especially in those whose corresponding normal samples have little to no expression of CRNDE. Further analysis of gene expression data sets of cancer cells with drug treatment demonstrated that anti-cancer drugs could efficiently repress the overexpression of CRNDE in cancer cell lines and patient samples. Finally, quantitative real-time PCR (qRT-PCR) assays by isoform-specific primers for 12 CRNDE spliced variants illustrated that CRNDE-g (previously defined CRNDE-h) was most abundant in the majority of cancer cell lines. Collectively, our findings demonstrate that CRNDE was commonly overexpressed in most types of cancer, emphasizing its role in tumorigenesis, and suggest that CRNDE with its distinct alternatively spliced isoforms might be promising biomarkers for cancer diagnosis and potential targets for cancer therapy.

Materials and methods

Collection of microarray gene expression data sets

The normalized expression levels of CRNDE across all normal human tissues and cell types were downloaded from the Body Atlas database of NextBio (http://www.nextbio.com) including 147 normal tissues and 173 cell types. Excluding the unassayed tissues and cell types, we finally retrieved the gene expression data from 109 normal tissues and 67 cell types. Cancer-associated microarray gene expression data sets were downloaded from the Gene Expression Omnibus (GEO) at the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov). Detailed information of various data sets is presented in Table 1.

To investigate the drug influence on CRNDE expression, we downloaded four gene expression data sets with drug treatment, encompassing three data sets on cancer cell lines and one data set on cancer patient samples. Two data sets were generated from the CRC line HCT116 (GSE15395) [23] and the prostate cancer cell line DU145 (GSE15392) [23] with the treatment of R547. One data set was generated from the AML cell line THP1 with the treatment of phorbol-12-myristate-13-acetate (PMA) (GSE46599) [24]. One data set was generated from nine chronic myeloid leukemia (CML) patients with the treatment of imatinib (GSE33075) [25].

Integration of gene expression microarrays

We integrated microarray data sets from the same cancer by reanalyzing their raw data and used robust multi-array average approach for normalization among different data sets. Subsequently, we pooled the patient samples and corresponding normal control samples for further comparative analysis of CRNDE expression.

Cell culture and reagents

Solid cancer cell lines including CRC cell lines HCT116 and HT29 cells and glioma tumor cell lines U251 and

Table 1 GEO data sets used to detect CRNDE expression in 15 types of cancers

Cancer type	Cancer name	GEO (No.)	Cancer samples (No.)	Control samples (No.)	Ref.
Solid tumors	CRC	GSE32323	CRC tissues (17)	Normal colorectal tissues[b] (17)	[27]
		GSE8671	Colorectal adenoma (32)	Normal colorectal mucosa[b] (32)	[28]
		GSE37364	CRC tissues (14)	Normal colonic mucosa (38)	[29]
		GSE4183	CRC and adenoma (30)	Normal controls (8)	[30]
	Glioma	GSE4290	Glioma samples (157)	Non-tumor samples (23)	[31]
	ACC	GSE19750	ACC samples (44)	Normal adrenal glands (4)	[32]
		GSE10927	ACC samples (33)	Normal adrenal cortex (10)	[33]
	Pancreatic cancer	GSE16515	Pancreatic tumor (36)	Normal pancreatic tissues (16)	[34]
		GSE22780	Pancreatic tumor (8)	Normal tissues[b] (8)	[35]
		GSE15471	PDAC samples (36)	Normal tissues[b] (36)	[36]
	Prostate cancer	GSE55945	Prostate cancer (12)	Normal tissues (7)	[37]
		GSE46602	Prostate cancer (36)	Normal prostate (14)	[38]
	Ovarian cancer	GSE14407	CEPI samples (12)	Normal OSE (12)	[39]
	Cervical cancer	GSE63514	Cervical cancer (28)	Normal cervical epithelium (24)	[40]
	NSCLC	GSE33532	NSCLC tissues (80)	Normal lung tissues (20)	[41]
	NMSC	GSE53462[a]	NMSC tissues (21)	Normal skin tissues (5)	[42]
Leukemia	ATL	GSE1466	ATL samples (41)	Normal T cells (3)	[43]
	AML	GSE12662	AML samples (76)	Normal PMN (5)	[44]
		GSE13159	AML samples (542)	Healthy BM (74)	[45,46]
	CML	GSE13159	CML samples (76)	Healthy BM (74)	[45,46]
	ALL	GSE13159	ALL samples (750)	Healthy BM (74)	[45,46]
	MDS	GSE13159	MDS samples (206)	Healthy BM (74)	[45,46]
	CLL	GSE13159	CLL samples (448)	Healthy BM (74)	[45,46]
		GSE67642[a]	CLL-CD19$^+$ cells (15)	Normal CD19$^+$ cells (9)	[47]
		GSE50006	CLL cells (188)	Normal B cells (32)	NA[c]
Total	15 types	21 data sets	2938	397	

[a]Data sets from Illumina HumanHT-12 v4.0 Expression BeadChip and others from Affymetrix HG-U133 arrays. [b]Pairs of normal and tumor tissue samples. [c]The reference is not available. BM, bone marrow. CEPI, ovarian cancer epithelia. OSE, ovarian surface epithelia. PDAC, pancreatic ductal adenocarcinoma.

U87MG cells were cultured in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Moregate Biotech, Bulimba, QLD, Australia). Leukemic cell lines including Kasumi-1, U937, THP1, and K562 cells were cultured in RPMI 1640 (Gibco) supplemented with 10% FBS. All cell lines were cultured at 37 °C in a humidified atmosphere with 5% CO_2 incubator.

qRT-PCR assays for 12 CRNDE transcripts in cancer cell lines

Total RNA from cancer cell lines was extracted using an RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Genomic DNA removal and RNA reverse transcription assays were performed with PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara, Otsu, Japan). qRT-PCR assays were performed using SYBR® Premix Ex Taq™ II (Takara, Otsu, Japan) on ABI ViiA 7 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The relative expression level of each gene was calculated as $2^{-[Ct(Gene)-Ct(GAPDH)]}$. Each assay was performed in triplicate. The isoform-specific primer pairs are listed in Table S1.

RT-PCR and agarose gel electrophoresis

RT-PCR assays were performed using KOD-Plus DNA Polymerase (Toyobo, Osaka, Japan) with 12 isoform-specific primers for CRNDE spliced transcripts (30 cycles at 94 °C for 15 s, at 58 °C for 30 s, and at 68 °C for 30 s). The PCR-amplified products of CRNDE transcripts were determined visually after performing electrophoresis on a 1% agarose gel containing GelRed™ Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA).

Statistics

Paired or unpaired *t*-test was used to validate the significance of the data.

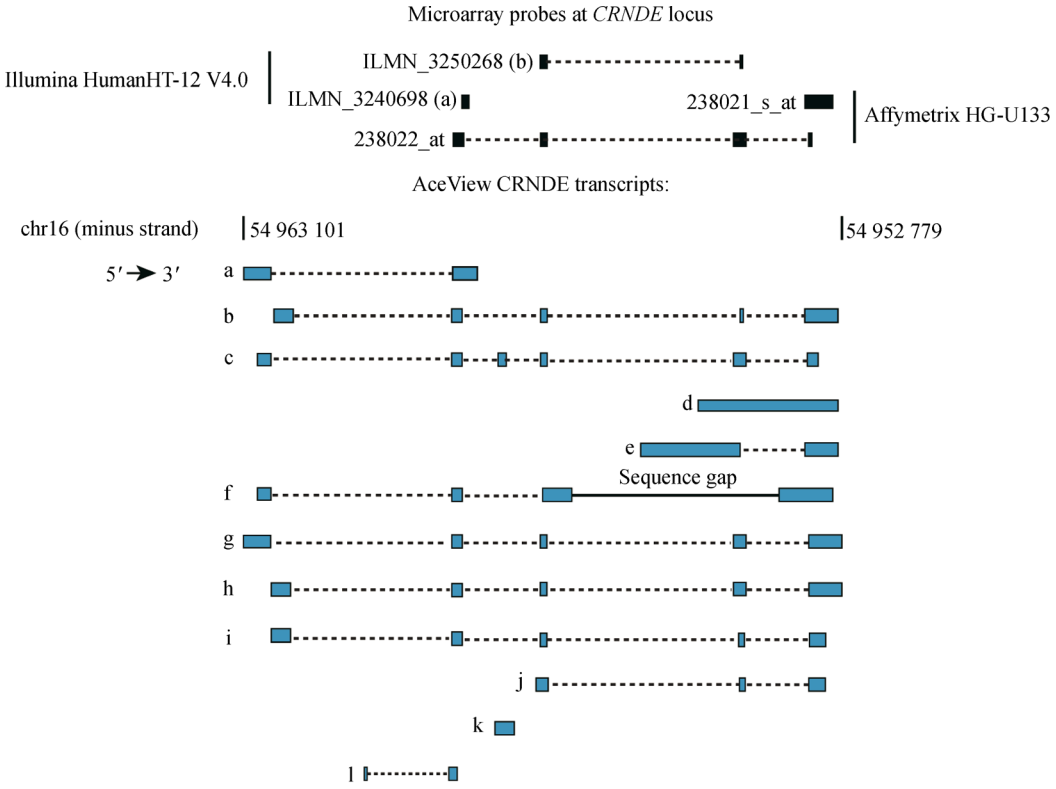

Fig. 1 Alternatively spliced variants derived from the *CRNDE* locus. The *CRNDE* genomic locus (on the reverse strand) and its 12 transcript variants are pictured. The blue boxes and black dotted lines represent exons and introns, respectively. The target regions by Illumina HumanHT-12 V4.0 (ILMN_3250268 and ILMN_3240698) and Affymetrix HG-U133 (238021_s_at and 238022_at) probes are shown on top of the schematic diagram. The sequence gap in CRNDE-f is the unknown sequence region.

Results

Genomic view of alternatively spliced variants on the *CRNDE* locus

As multiple alternatively spliced variants of CRNDE have been reported in literature [14,26], we first updated the possible variants using the latest human NCBI AceView database. As shown in Fig. 1, the *CRNDE* genomic locus, spanning from 54 952 779 to 54 963 101 on human chromosome 16q based on the Human Genome Assembly GRCh37/hg19, produces at least 12 different transcript variants. These 12 variants include 7 alternatively spliced variants (CRNDE-a, -b, -c, -g, -h, -i, and -j), 3 partially spliced variants (CRNDE-e, -f, and -l), and 2 unspliced isoforms (CRNDE-d and -k).

Although gene expression microarrays are commonly designed to detect mRNA expression levels, several microarray platforms also contain a fraction of lncRNAs. Certain microarray platforms even have the ability to assess the expression of alternatively spliced isoforms, at least to some extent, due to multiple probes designed to cover the distinct genomic locus. After screening the widely used microarray platforms, we found that two microarray platforms, i.e., the Affymetrix HG-U133 array and Illumina HumanHT-12 v4.0 Expression BeadChip, contained the probes specific for CRNDE. The Affymetrix HG-U133 array contains two CRNDE probes, i.e., 238021_s_at and 238022_at. The 238021_s_at probe targets the 3′-terminal exon and thus can detect the majority of the CRNDE isoforms containing exon 6, whereas the 238022_at probe is designed across exons 2, 4, 5, and 6, and thus only detects the exonic isoforms of CRNDE (Fig. 1). The Illumina HumanHT-12 v4.0 Expression BeadChip also contains two probes, i.e., ILMN_3240698 and ILMN_3250268, which specifically target the CRNDE-a and CRNDE-b transcripts, respectively (Fig. 1). These available probes may reflect, at least to some extent, the expression levels of CRNDE alternatively spliced isoforms.

Collection of CRNDE probe-containing microarrays in normal and cancer cells

To assess CRNDE expression in normal tissues/cell types and various cancer types, we retrieved the expression data from two large repositories of gene expression data. The expression data in normal tissues and cell types were

extracted from Body Atlas. We retrieved the gene expression data from 109 normal tissues and 67 normal cell types. The gene expression data of various kinds of cancer samples and corresponding normal or adjacent noncancerous controls were retrieved from the GEO database. We retrieved 21 data sets of the gene expression data encompassing 15 cancer types, i.e., 9 solid tumors and 6 hematopoietic cancers, in which 2938 patient samples and 397 corresponding control samples were included. Among these data sets, 16 were generated from solid tumors including 4 for CRC, 1 for glioma, 2 for adrenocortical carcinoma, 3 for pancreatic tumor, 2 for prostate cancer, and 1 for ovarian adenocarcinoma, cervical cancer, non-small cell lung cancer (NSCLC), and non-melanoma skin cancer (NMSC) specimens. Additional five data sets were generated from hematopoietic malignancies including two for chronic lymphocytic leukemia (CLL), one for AML, one for adult T cell leukemia (ATL), and one for five types of hematopoietic cancers containing AML, CML, acute lymphocytic leukemia (ALL), CLL, and myelodysplastic syndrome (MDS). Detailed information about the data sets of cancer samples and corresponding normal controls is presented in Table 1.

CRNDE expression showing a tissue- and cell-specific pattern in normal tissues/cells

According to the annotation from the Body Atlas at the NextBio platform, 109 normal tissue types and 67 cell types were both classified into 10 systems. Additionally, we further ranked 10 systems and their corresponding tissues/cell types from relatively higher expression to lower expression of CRNDE. CRNDE was highly expressed in the urogenital, exocrine, integumentary, and musculoskeletal system, in which the expression levels of CRNDE in almost corresponding tissues were higher than the median expression level in total tissues, such as testes, parotid gland, and skin (Fig. 2). Furthermore, we also found that CRNDE was highly expressed in corresponding cell types, such as spermatozoa, ovary, and various types of prostate and breast cells (Fig. S1). Besides, CRNDE was expressed at highest levels in dental odontoblasts of the digestive system among all tested cells (Fig. S1). It was slightly expressed in most tissues of the immune system and endocrine system, such as spleen, bone marrow, and omental adipose tissue (Fig. 2). Correspondingly, we found that the expression level of CRNDE was lowest in mononuclear cells and B/T lymphocytes of peripheral blood and also in neutrophils of bone marrow. Moreover, the expression level of CRNDE in colonic epithelial cells of the digestive system was also lower than the median expression level of all cells (Fig. S1). The above results demonstrated that CRNDE was expressed in a tissue- and cell-specific pattern.

CRNDE overexpression in the majority of detected solid cancers

To investigate whether deregulated CRNDE expression is common in cancer, we first assessed the expression levels of CRNDE in solid tumors. Among these nine different solid cancer types, we observed that CRNDE was obviously overexpressed in six of nine solid tumors, with three reported, CRC, glioma, and pancreatic tumors, and three unreported, adrenocortical carcinoma, prostate cancer, and NMSC (Fig. 3A–3F and Fig. S2A–S2F). In this study, we integrated multiple gene expression data sets from various platforms and patient samples and further verified the overexpression of CRNDE in these three reported tumors. In addition, we found the expression level of CRNDE was significantly downregulated in ovarian cancer (Figs. 3G and S2G) and exerted no difference in cervical cancer and NSCLC, compared with the corresponding normal cervical epithelium cells and normal lung tissues (Fig. 3H and 3I and Fig. S2H and S2I).

Furthermore, expression levels of CRNDE were detected by four probes from two microarray platforms, which partially represented different alternatively spliced isoforms of CRNDE. As shown in Fig. 3 and Fig. S2, the corresponding two probes presented similar expression patterns except in prostate cancer. The expression levels of CRNDE detected by 238021_s_at probe revealed higher expression in prostate cancer tissues than normal control samples (Fig. 3E); however, the expression levels of fully spliced CRNDE isoforms detected by 238022_at probe had no difference between prostate cancer tissues and normal control samples (Fig. S2E). These results indicated that CRNDE and its distinct spliced isoforms were overexpressed in the majority of the detected cancer types and expressed in a cancer-specific pattern.

Highly expressed CRNDE in many types of hematopoietic cancers

To further investigate the expression patterns of CRNDE in hematologic malignancies, we assessed CRNDE expression in five gene expression data sets of hematologic cancers (Table 1). We first compared the expression of CRNDE between 750 ALL patient samples, 448 CLL patient samples, 542 AML patient samples, 76 CML patient samples, 206 MDS patient samples with 74 nonleukemia and healthy bone marrow samples. As shown in Fig. 4A, we found that CRNDE was significantly upregulated in ALL, AML, CML and MDS, and downregulated in CLL. We further verified the overexpression pattern of CRNDE in AML by analyzing another data set (GSE12662) (Fig. S3). We compared CRNDE expression from 76 AML patient samples, five normal polymorphonuclear neutrophils (PMNs), and five purified normal

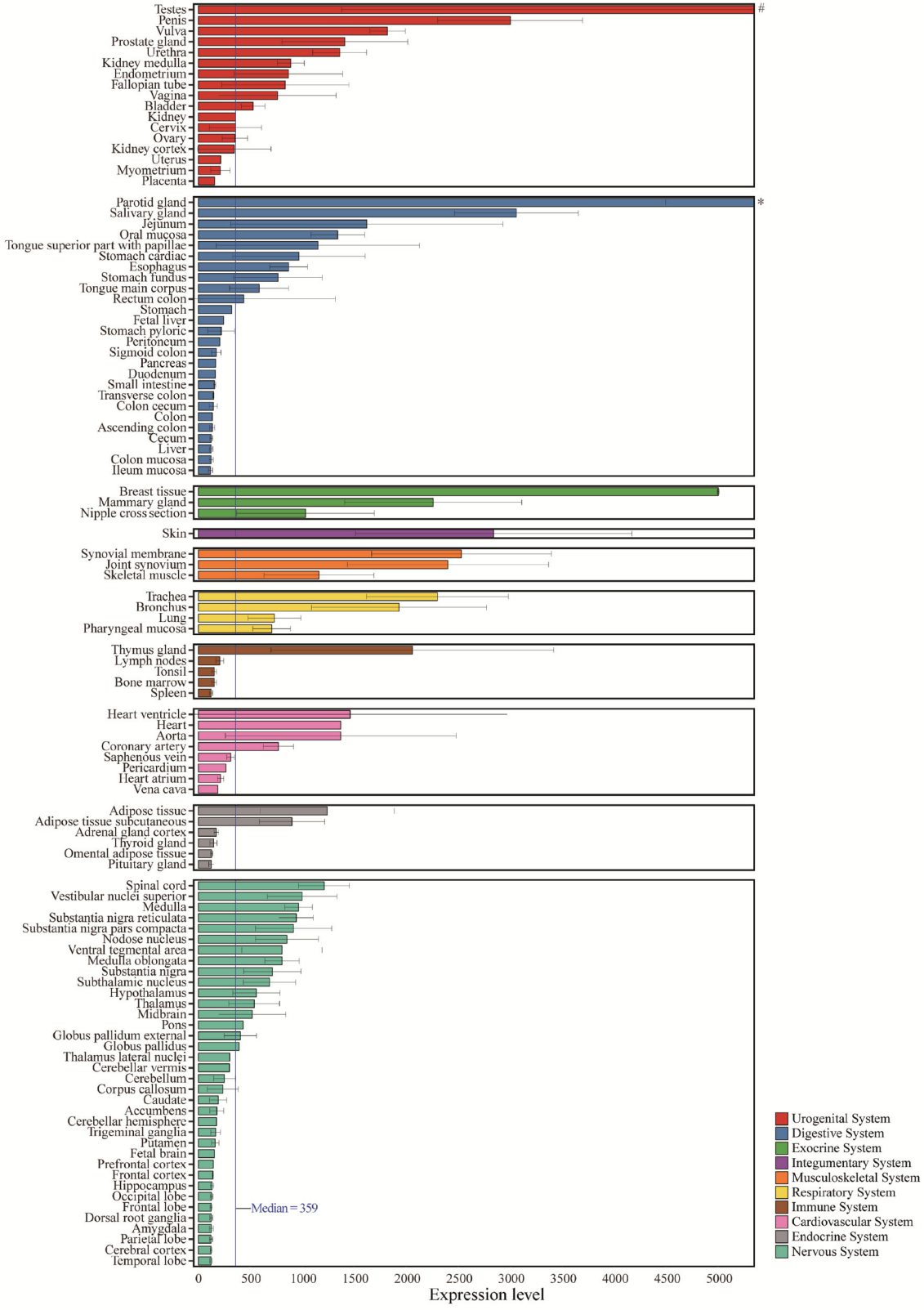

Fig. 2 The tissue-specific expression pattern of CRNDE in various normal human tissues. The expression data of CRNDE in 109 types of normal tissues are from the Body Atlas, Tissues, at http://www.nextbio.com. The unassayed tissues have been omitted. The median expression level of total tissue types is shown with the blue line. # stands for the expression value of 16 700 in testes and * stands for 6020 in the parotid gland. The data are presented as mean ± standard deviation.

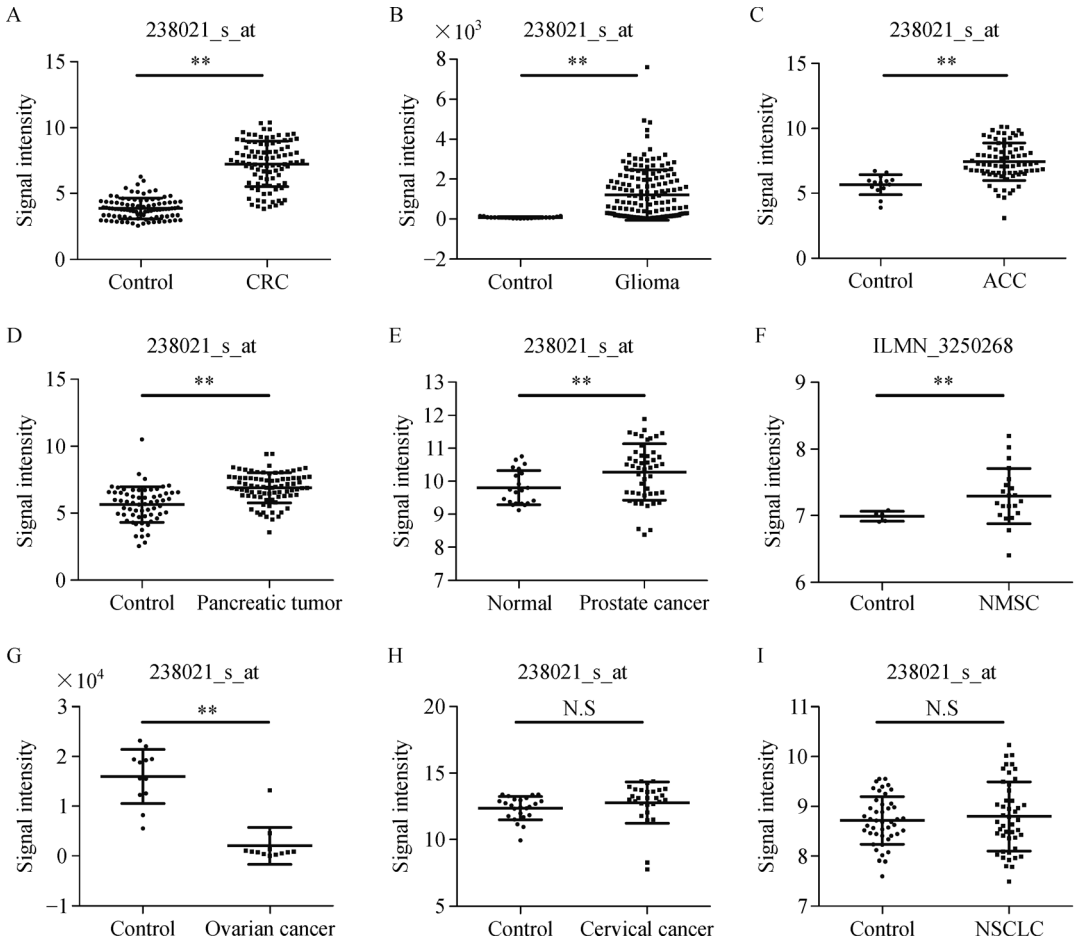

Fig. 3 Cancer-specific expression patterns of CRNDE in solid tumors. Sixteen microarray data sets were analyzed, encompassing nine types of solid tumors with 596 tumor samples and 274 corresponding normal or adjacent noncancerous controls. The signal intensities of CRNDE detected by 238021_s_at probe are presented by scatter plots in CRC (A), glioma (B), ACC (C), pancreatic tumor (D), prostate cancer (E), ovarian cancer (G), cervical cancer (H), and NSCLC (I). The signal intensity of CRNDE in NMSC was detected by ILMN_3250268 probe (F). The detailed information of the examined data sets can be found in Table 1. Paired or unpaired *t*-test was performed to analyze significant differences (* $P < 0.05$, ** $P < 0.01$).

myeloid precursor $CD34^+$ cells. Besides highly expressed in AML, we also found that CRNDE was moderately expressed in $CD34^+$ cells than in PMNs cells (Fig. S3A). These results indicated that CRNDE expression appeared higher at the early stages of myeloid development and downregulated during differentiation, which may emphasize its temporal expression patterns. In addition, we analyzed the differential expression pattern of CRNDE between 41 ATL samples and 3 normal $CD4^+$ T cells. As illustrated in Fig. 4B, the expression of CRNDE was also higher in ATL samples than in normal cells. Furthermore, the two CRNDE-specific probes from HG-U133 produced consistent expression patterns.

Interestingly, the expression levels of CRNDE in two additional CLL-associated data sets (GSE67642 and GSE50006) showed differential expression detected by four probes, which partially represented spliced isoforms of CRNDE to some extent. As shown in Fig. S3B and S3C, the total expression of CRNDE represented by the 238021_s_at probe from HG-U133 was lower in CLL samples. However, the expression of CRNDE isoforms (fully spliced) detected by the 238022_at probe showed no difference between CLL and normal B cells. Likewise, the CRNDE-a isoform detected by ILMN_3240698 probe showed lower expression in CLL samples, but the CRNDE-b isoform detected by ILMN_3250268 showed no difference between CLL and normal B cells. The data implicated that the expression of 12 CRNDE spliced isoforms differed in CLL.

Taken together, CRNDE was also upregulated in the majority of leukemia. The differential expression pattern of CRNDE and its isoforms in leukemia might be the characteristic of distinct subtypes of leukemia/subtype-specific expression pattern in leukemia.

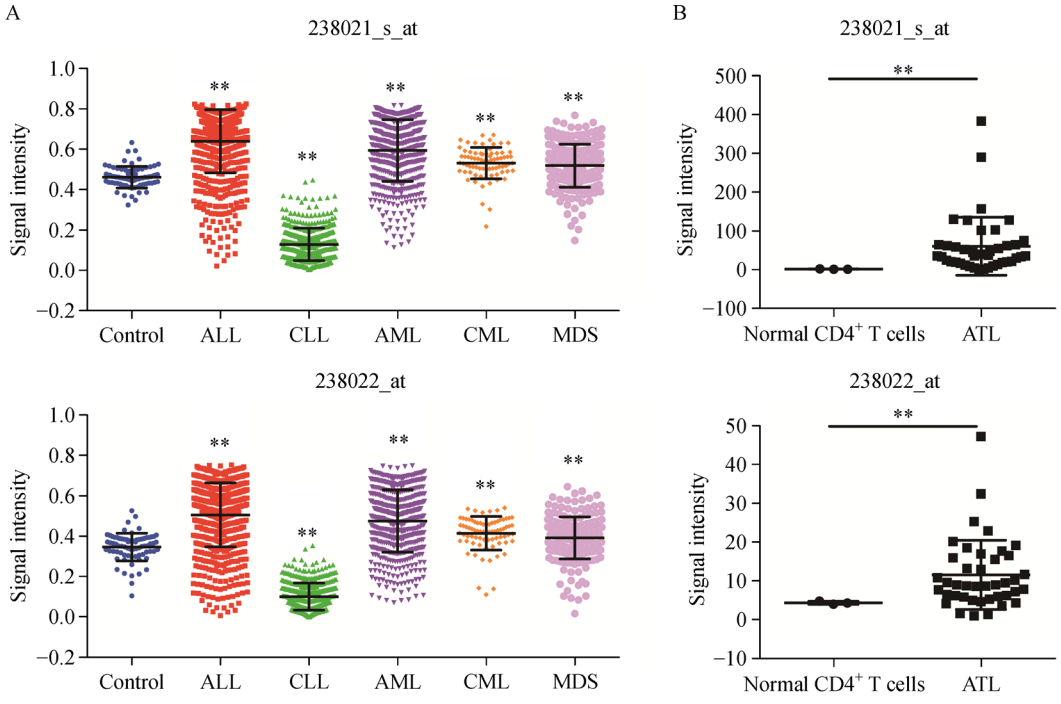

Fig. 4 Highly expressed CRNDE in the majority of hematopoietic cancers. (A) The microarray data (GSE13159) included 5 types of leukemia encompassing 2022 leukemia samples and 74 corresponding normal controls. The signal intensities of CRNDE expression are shown by scatter plots and detected by 238021_s_at (upper panel) and 238022_at (lower panel) probes. The *x*-axis represents the five types of indicated leukemia. (B) The expression levels of CRNDE in ATL patient samples were compared with normal CD4-positive T cells by 238021_s_at (upper panel) and 238022_at (lower panel) probes. Unpaired *t*-test was performed to analyze significant differences (* $P < 0.05$, ** $P < 0.01$).

Repression of overexpressed CRNDE by anti-cancer drugs in cancer cell lines and patient samples

The overexpression of CRNDE in multiple cancers suggested its potential oncogenic activity in tumorigenesis. We wondered whether anti-cancer drugs could correct the abnormally high expression of CRNDE in cancers. Therefore, we downloaded four gene expression data sets with drug treatment, encompassing three data sets on cancer cell lines and one data set on CML patient samples. We first investigated the drug effects on CRNDE expression in solid cancer cell lines, i.e., the colon cancer cell line HCT116 and the prostate cancer cell line DU145. The examined drug is R547, a novel cyclin-dependent kinase selective inhibitor in phase I clinical trials, which has shown a potent anti-growth effect on proliferating cancer cells [48]. We compared the intensity of CRNDE-specific probes in HCT116 and DU145 cell lines with human peripheral blood mononuclear (PBMC) cells [23]. The PBMC cells were considered as the nonproliferating cells for the control. As illustrated in Fig. 5A, the intensity of CRNDE in HCT116 and DU145 was significantly higher compared with the intensity of CRNDE in PBMC cells, which was consistent with our previous results in CRC and prostate cancer and further emphasized its role in promoting proliferation of cancers. Subsequently, we compared the intensity of CRNDE-specific probes in HCT116 cells and found different extents of reduction between two CRNDE-specific probes after R547 treatment. Especially, the intensity of 238021_s_at showed greater reduction than that of 238022_at (Fig. 5B). These results indicated that CRNDE isoforms presented different responses to drug treatment. Though CRNDE was higher in DU145 cells, we found that the intensities of two CRNDE-specific probes exhibited no difference after R547 treatment (Fig. 5C). These results in solid tumors indicated that CRNDE might display cancer-specific drug response, which may be due to the distinct genetic characteristics of different cancers.

Subsequently, we examined the influences of anti-cancer drugs on CRNDE expression in leukemia cells. We detected the drug response of CRNDE in M5 subtype of AML cell line THP1 cells, which is arrested at monocyte stage of macrophage differentiation. PMA is a common drug to induce the terminal monocyte–macrophage differentiation and can promote differentiation of THP1 leukemic cells into macrophage-like cells. We first compared the intensities of CRNDE between THP1 and

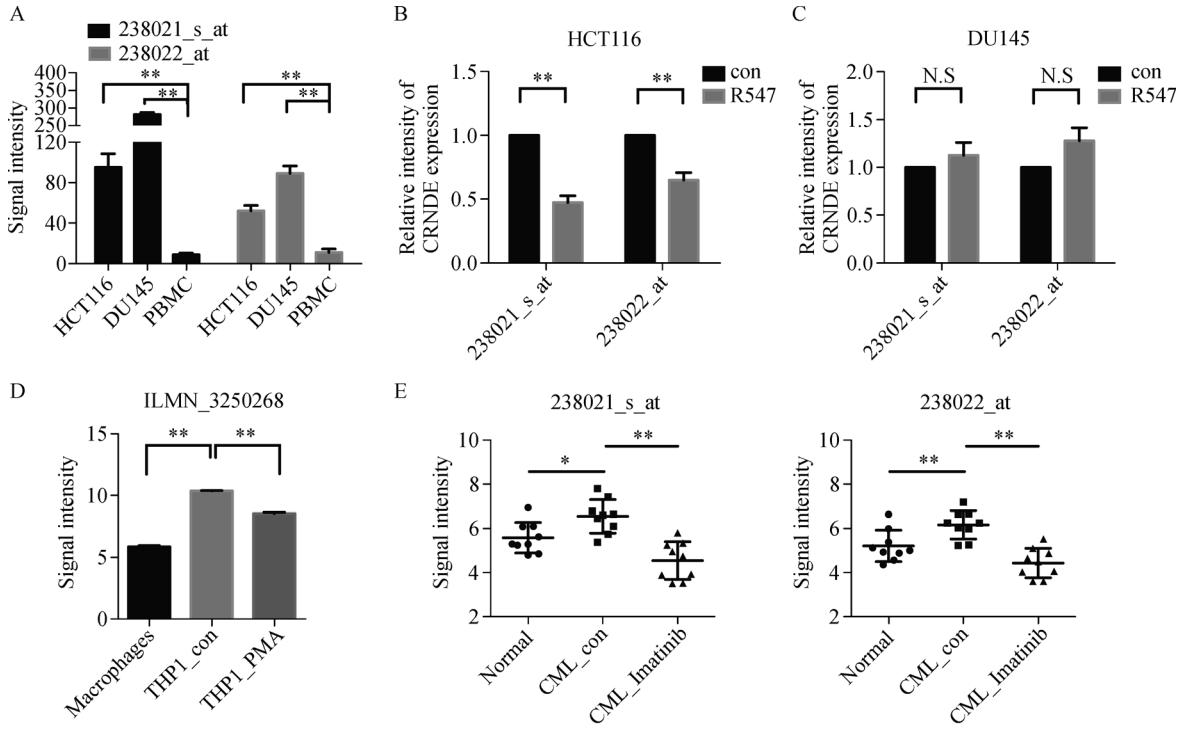

Fig. 5 Repression of overexpressed CRNDE by anti-cancer drugs in cancer cell lines and patient samples. (A) The signal intensities of CRNDE expression levels were detected by two Affymetrix HG-U133 probes, 238021_s_at and 238022_at, in HCT116 cells (GSE15395), DU145 cells (GSE15392), and PBMC cells (GSE15389). (B and C) The relative intensities of CRNDE expression were detected by 238021_s_at and 238022_at probes in HCT116 (B) and DU154 (C) before and after R547 treatment, respectively [23]. (D) CRNDE expression was detected by ILMN_3250268 probe in macrophages and AML-M5 cell line THP1 with PMA treatment (GSE46599) [24]. (E) The signal intensities of CRNDE were detected by 238021_s_at and 238022_at probes in normal bone marrow samples and CML patient samples before and after imatinib therapy, respectively (GSE33075) [25]. Paired or unpaired *t*-test was performed to analyze significant differences (* $P < 0.05$, ** $P < 0.01$).

macrophage cells. The expression of CRNDE was obviously higher in THP1 than in macrophage cells (Fig. 5D). These results also further verified our previous results that the expression of CRNDE was higher in abnormal proliferating leukemic cells than normal cells. Subsequently, we compared the intensities of CRNDE in THP1 cells before and after PMA treatment and discovered that PMA could significantly downregulate the expression of CRNDE (Fig. 5D). Besides AML, we further found that the expression of CRNDE was significantly downregulated in CML patient samples after imatinib therapy (Fig. 5E). CML is also a clonal myeloproliferative disorder characterized by the aberrant expression of the BCR/ABL fusion oncogene in accumulated myeloid precursor cells. As the standard therapy for newly diagnosed CML patients, imatinib can specifically target and degrade BCR/ABL fusion protein to cure patients. We compared the expression between CML patient samples and normal cells from healthy donors. Likewise, the expression of CRNDE was indeed significantly higher in CML patient samples (Fig. 5E), which emphasized its aberrant overexpression in hematopoietic cancer and its potential roles in leukemogenesis and differentiation therapy.

Experimental evidence illustrating the detailed expression patterns of 12 alternatively spliced isoforms of CRNDE in eight types of cancer cell lines

Although microarray-based profiles provide gene expression data on a large number of patients across a rich resource of cancer types, detailed gene expression information on alternatively spliced isoforms, especially for CRNDE with at least 12 isoforms, is limited. To investigate the exact expression pattern on each isoform of CRNDE in different types of cancer, we performed qRT-PCR to detect the expression of each isoform in several cell lines, which were shown to have overexpressed CRNDE. We first designed isoform-specific primers to distinguish the 12 isoforms. The principles of primer design were mainly according to their different splicing sites or distinguished sequence in retained introns or exons (Fig. 6A), which were further confirmed by sequencing each fragment amplified by corresponding primer pairs (Fig. 6B).

We detected the expression of CRNDE isoforms on eight types of cancer cell lines, encompassing two CRC cell lines (HT29 and HCT116), two glioma cell lines

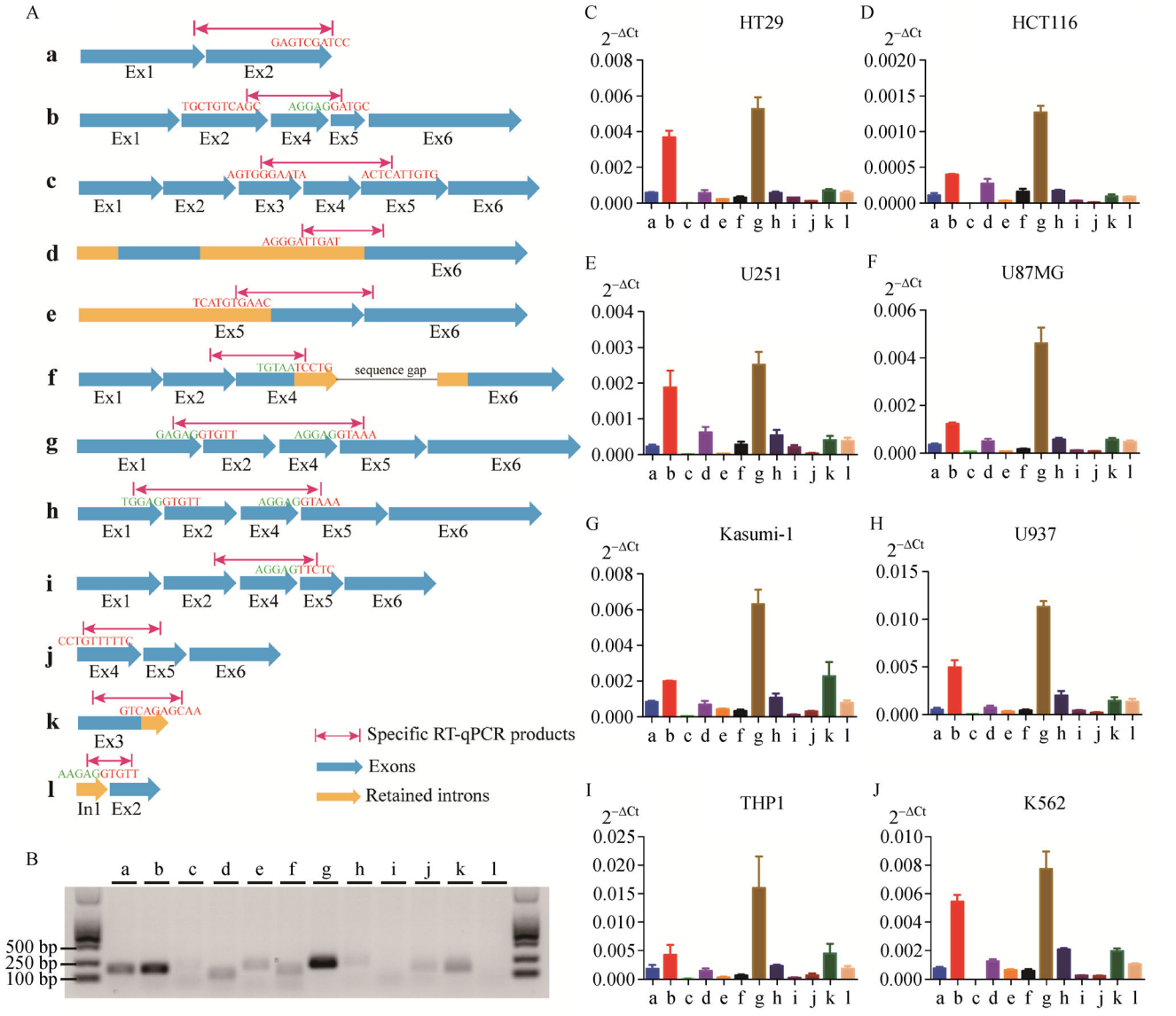

Fig. 6 Experimental evidence illustrating the detailed expression patterns of 12 alternatively spliced isoforms of CRNDE in eight types of cancer cell lines. (A) The schematic diagram shows the CRNDE transcripts and fragments amplified by isoform-specific primer pairs. The blue and orange arrows represent exons and retained introns, respectively. The amplified fragments are marked with bidirectional red arrows. Unique sequences for discriminating each isoform are listed above the corresponding isoforms. (B) RT-PCR assays were performed to detect each isoform of CRNDE by corresponding specific primers. In addition, the amplified fragments were visualized by electrophoresis on agarose gels. (C–J) The relative expression of 12 CRNDE isoforms were determined by qRT-PCR assays in CRC cell lines HT29 (C) and HCT116 cells (D), glioma cancer cell lines U251 (E) and U87MG cells (F), AML cell lines Kasumi-1 (G), U937 (H) and THP1 cells (I), and CML cell line K562 cells (J). The *x*-axis represents 12 isoforms of CRNDE by letters. The *y*-axis shows the mean $2^{-\Delta Ct}$ values from three independent experiments, and error bars represent the standard deviation.

(U251 and U87MG), three AML cell lines (U937, Kasumi-1, and THP1), and one CML cell line (K562). As shown in Fig. 6C–6J, we found that the CRNDE-g isoform was the most overexpressed isoform in examined cancer cell lines. Our results underscored the potential roles of CRNDE-g in the tumorigenesis. We also found that CRNDE-a, -b, -d, -f, -h, -k, and -l were differentially expressed in these cell lines. Especially, CRNDE-b was also obviously over-expressed in cancer cells, only modestly lower than CRNDE-g. These differentially expressed CRNDE isoforms may account for the characteristics of cancer-specific expression patterns.

Discussion

lncRNAs are emerging as key regulators of diverse biological functions in cell development and tumorigenesis.

Alternative splicing mechanisms generate various lncRNA isoforms, which increases the diversity of function and transcriptional regulation mechanisms of lncRNA. In this study, we demonstrated that the lncRNA CRNDE was expressed in a tissue- and cancer-specific pattern and commonly upregulated in multiple cancers by analyzing thousands of cancer patients. Additionally, anti-cancer drugs could efficiently repress the aberrant high expression of CRNDE. Furthermore, we found that CRNDE-g was the most abundant isoform in cancer cell lines, which implied that CRNDE with its distinct spliced isoforms could be the characteristic of various cancer types and the potential targets for cancer therapy.

As lncRNA CRNDE has been initially identified and investigated widely in CRC, more attention has been paid to CRNDE. Thus, its aberrant expression and potential oncogenic role in tumorigenesis prompt us to determine whether CRNDE expression is commonly altered in different cancer types. Our studies retrieved 21 gene expression data encompassing thousands of patient samples in 15 cancer types. Through our reanalysis of these data sets, we newly identified that CRNDE was significantly highly expressed in prostate cancer, ACC, NMSC, AML, ATL, CML, ALL, and MDS, besides previously reported CRC, glioma, and pancreatic cancer. Recent studies have suggested that the overexpression of CRNDE may be related to its upstream deregulation by several aspects. For the same promoter shared with IRX5, CRNDE is positively associated with IRX5 expression in CRC [21]. Additionally, IRX5 is also reported to be overexpressed in multiple cancers and exert its oncogenic effects by regulating target genes and cell apoptosis [49]. Our analysis also demonstrated the partially concordant expression pattern between CRNDE and IRX5 (data not shown). However, the real mechanism of the correlation between CRNDE and IRX5 remains poorly understood. Furthermore, we also demonstrated that CRNDE was highly upregulated in hematopoietic cancers. In addition to the upregulation of CRNDE in cancers, we also found that it can be downregulated in ovarian cancer and CLL. The evidence is showing that the decreased expression of CRNDE is associated with the accumulation of TP53 [50]. Moreover, the downregulated CRNDE is considered to be a potential biomarker for poor prognostic evaluation in women with ovarian cancers. Furthermore, anti-cancer drugs, especially anti-proliferating and differentiation therapy, can efficiently correct the aberrant expression of CRNDE. The above results suggest that CRNDE may be a potential target for cancer therapy.

CRNDE is a multi-functional lncRNA that exerts various oncogenic functions in tumorigenesis. The upregulation of CRNDE can activate mTOR signaling, insulin/IGF signaling, and Wnt/β-catenin signaling pathways and inhibit cell apoptosis, thus promoting abnormal cell proliferation, metastasis, and invasion in many cancers [19,26,51]. Several studies have revealed the preliminary transcriptional mechanism of CRNDE in the regulation of its tumor-promoting roles. First, CRNDE can act as competing endogenous RNA and microRNA sponges to regulate downstream signaling pathways. For example, CRNDE can competitively bind to miR-384 and negatively regulate its expression and tumor-suppressive function, primarily regulating its repressed gene, piwi-like RNA-mediated gene silencing 4 [52]. Besides, CRNDE can bind to and negatively regulate miR-186 expression and consequently repress its downstream signaling pathways [20]. CRNDE also can sponge miR-181a-5p to mediate Wnt/β-catenin signaling pathway and miR-136 to promote metastasis and oxaliplatin resistance in CRC [18,53]. Furthermore, CRNDE can bind polycomb repressive complex 2 and CoREST chromatin-modifying complexes in the nucleus, through epigenetic mechanism to modulate target genes [54]. Although these preliminary results have explained the mechanism of CRNDE regulation and functions, many issues need to be investigated in the future.

Alternative pre-mRNA splicing can generate functionally distinct mRNAs by splicing exons of pre-mRNA in different arrangements [2]. As a multiexonic lncRNA, CRNDE undergoes extensive alternative splicing to produce at least 12 isoforms according to the latest human NCBI AceView database. According to various amounts of unspliced introns and exons, these isoforms are classified into three main patterns, fully spliced introns (CRNDE-a, -b, -c, -g, -h, -i, and -j), retained intronic sequence (CRNDE-e, -f, and -l) and unspliced isoforms (CRNDE-d and -k). These isoforms predict that CRNDE may exert diverse roles in cell development and tumorigenesis. The transcript abundance profile illustrates that CRNDE-d is the dominant transcript in normal colorectal tissue, whereas, in colorectal adenomas and cancers, the expression of CRNDE-d decreases, and other CRNDE spliced variants may be upregulated [14]. These findings suggest that CRNDE-d may participate in normal colorectal development. Thus, to elucidate the distinct function of CRNDE involving multiple cancer types, the exact expression pattern of various isoforms in different cancers needs to be identified. Our results revealed that CRNDE-g was the most abundant transcript in all detected cancer cell lines. Recently, it has been gaining attention due to its oncogenic effects and clinical implementation in CRC. Overexpression and knockdown assays demonstrate that CRNDE-g is involved in the regulation of aberrant cell proliferation [21,51]. Moreover, it has been used to efficiently distinguish adenoma with normal mucosa and is significantly correlated with metastasis and prognosis in CRC [21]. Exosomal CRNDE-g is easily detected from the serum of patient samples. Thus, CRNDE-g can be considered as a biomarker for diagnosis and prognosis for CRC [22]. In addition, CRNDE-b can produce 84 aa

nuclear peptide named CRNDEP, which has been reported to contribute to the regulation of cell proliferation and promote stress granules' formation in HeLa cells [55]. Our studies further manifest the clinical significance of CRNDE and its specific isoform in multiple cancers, not only solid but also hematopoietic cancers.

Therefore, our results put forward CRNDE and its specific isoforms as sensitive and specific molecular markers for distinct cancer types for their characteristics of cancer-specific expression pattern. Furthermore, their potential oncogenic functions in tumorigenesis are also implied to be promising targets for cancer therapy, which may be further investigated in the future.

Acknowledgements

This work was supported in part by National Natural Science Foundation of China (Nos. 81530003, 81300403, 81770153 and 91440114), The National Key Research and Development Program (No. 2016YFC0902800), Shanghai Leading Talent Projects (No. 2015008) and the Academic Leader Program of Shanghai Science and Technology Committee (No. 2015137).

Compliance with ethics guidelines

Xuefei Ma, Wei Zhang, Rong Zhang, Jingming Li, Shufen Li, Yunlin Ma, Wen Jin, and Kankan Wang declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-017-0557-0 and is accessible for authorized users.

References

1. Zhang Z, Pal S, Bi Y, Tchou J, Davuluri RV. Isoform level expression profiles provide better cancer signatures than gene level expression profiles. Genome Med 2013; 5(4): 33
2. Miura K, Fujibuchi W, Unno M. Splice isoforms as therapeutic targets for colorectal cancer. Carcinogenesis 2012; 33(12): 2311–2319
3. Gellert P, Ponomareva Y, Braun T, Uchida S. Noncoder: a web interface for exon array-based detection of long non-coding RNAs. Nucleic Acids Res 2013; 41(1): e20
4. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev 2009; 23(13): 1494–1504
5. Kornienko AE, Dotter CP, Guenzl PM, Gisslinger H, Gisslinger B, Cleary C, Kralovics R, Pauler FM, Barlow DP. Long non-coding RNAs display higher natural expression variation than protein-coding genes in healthy humans. Genome Biol 2016; 17(1): 14
6. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464(7291): 1071–1076
7. Wapinski O, Chang HY. Long noncoding RNAs and human disease. Trends Cell Biol 2011; 21(6): 354–361
8. Liu Y, Zhang M, Liang L, Li J, Chen YX. Over-expression of lncRNA DANCR is associated with advanced tumor progression and poor prognosis in patients with colorectal cancer. Int J Clin Exp Pathol 2015; 8(9): 11480–11484
9. Ren YK, Xiao Y, Wan XB, Zhao YZ, Li J, Li Y, Han GS, Chen XB, Zou QY, Wang GC, Lu CM, Xu YC, Wang YC. Association of long non-coding RNA HOTTIP with progression and prognosis in colorectal cancer. Int J Clin Exp Pathol 2015; 8(9): 11458–11463
10. Díaz-Beyá M, Brunet S, Nomdedéu J, Pratcorona M, Cordeiro A, Gallardo D, Escoda L, Tormo M, Heras I, Ribera JM, Duarte R, de Llano MP, Bargay J, Sampol A, Nomdedeu M, Risueño RM, Hoyos M, Sierra J, Monzo M, Navarro A, Esteve J; Cooperative AML group CETLAM. The lincRNA HOTAIRM1, located in the HOXA genomic region, is expressed in acute myeloid leukemia, impacts prognosis in patients in the intermediate-risk cytogenetic category, and is associated with a distinctive microRNA signature. Oncotarget 2015; 6(31): 31613–31627
11. Joo JH, Ryu D, Peng Q, Sugrue SP. Role of Pnn in alternative splicing of a specific subset of lncRNAs of the corneal epithelium. Mol Vis 2014; 20: 1629–1642
12. Tilgner H, Knowles DG, Johnson R, Davis CA, Chakrabortty S, Djebali S, Curado J, Snyder M, Gingeras TR, Guigó R. Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs. Genome Res 2012; 22(9): 1616–1625
13. Massone S, Vassallo I, Castelnuovo M, Fiorino G, Gatta E, Robello M, Borghi R, Tabaton M, Russo C, Dieci G, Cancedda R, Pagano A. RNA polymerase III drives alternative splicing of the potassium channel-interacting protein contributing to brain complexity and neurodegeneration. J Cell Biol 2011; 193(5): 851–866
14. Graham LD, Pedersen SK, Brown GS, Ho T, Kassir Z, Moynihan AT, Vizgoft EK, Dunne R, Pimlott L, Young GP, Lapointe LC, Molloy PL. Colorectal Neoplasia Differentially Expressed (CRNDE), a novel gene with elevated expression in colorectal adenomas and adenocarcinomas. Genes Cancer 2011; 2(8): 829–840
15. Fu XL, Liu DJ, Yan TT, Yang JY, Yang MW, Li J, Huo YM, Liu W, Zhang JF, Hong J, Hua R, Chen HY, Sun YW. Analysis of long non-coding RNA expression profiles in pancreatic ductal adenocarcinoma. Sci Rep 2016; 6(1): 33535
16. Zhang X, Sun S, Pu JK, Tsang AC, Lee D, Man VO, Lui WM, Wong ST, Leung GK. Long non-coding RNA expression profiles predict clinical phenotypes in glioma. Neurobiol Dis 2012; 48(1): 1–8
17. Ellis BC, Molloy PL, Graham LD. CRNDE: a long non-coding RNA involved in cancer, neurobiology, and development. Front Genet 2012; 3: 270
18. Han P, Li JW, Zhang BM, Lv JC, Li YM, Gu XY, Yu ZW, Jia YH, Bai XF, Li L, Liu YL, Cui BB. The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/β-catenin signaling. Mol Cancer 2017; 16(1): 9
19. Shao K, Shi T, Yang Y, Wang X, Xu D, Zhou P. Highly expressed

lncRNA CRNDE promotes cell proliferation through Wnt/β-catenin signaling in renal cell carcinoma. Tumour Biol 2016; 37(12): 15997–16004

20. Zheng J, Li XD, Wang P, Liu XB, Xue YX, Hu Y, Li Z, Li ZQ, Wang ZH, Liu YH. CRNDE affects the malignant biological characteristics of human glioma stem cells by negatively regulating miR-186. Oncotarget 2015; 6(28): 25339–25355
21. Liu T, Zhang X, Yang YM, Du LT, Wang CX. Increased expression of the long noncoding RNA CRNDE-h indicates a poor prognosis in colorectal cancer, and is positively correlated with IRX5 mRNA expression. Onco Targets Ther 2016; 9: 1437–1448
22. Liu T, Zhang X, Gao S, Jing F, Yang Y, Du L, Zheng G, Li P, Li C, Wang C. Exosomal long noncoding RNA CRNDE-h as a novel serum-based biomarker for diagnosis and prognosis of colorectal cancer. Oncotarget 2016; 7(51): 85551–85563
23. Berkofsky-Fessler W, Nguyen TQ, Delmar P, Molnos J, Kanwal C, DePinto W, Rosinski J, McLoughlin P, Ritland S, DeMario M, Tobon K, Reidhaar-Olson JF, Rueger R, Hilton H. Preclinical biomarkers for a cyclin-dependent kinase inhibitor translate to candidate pharmacodynamic biomarkers in phase I patients. Mol Cancer Ther 2009; 8(9): 2517–2525
24. Goujon C, Moncorgé O, Bauby H, Doyle T, Ward CC, Schaller T, Hué S, Barclay WS, Schulz R, Malim MH. Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. Nature 2013; 502(7472): 559–562
25. Benito R, Lumbreras E, Abáigar M, Gutiérrez NC, Delgado M, Robledo C, García JL, Rodríguez-Vicente AE, Cañizo MC, Rivas JM. Imatinib therapy of chronic myeloid leukemia restores the expression levels of key genes for DNA damage and cell-cycle progression. Pharmacogenet Genomics 2012; 22(5): 381–388
26. Ellis BC, Graham LD, Molloy PL. CRNDE, a long non-coding RNA responsive to insulin/IGF signaling, regulates genes involved in central metabolism. Biochim Biophys Acta 2014; 1843(2): 372–386
27. Khamas A, Ishikawa T, Shimokawa K, Mogushi K, Iida S, Ishiguro M, Mizushima H, Tanaka H, Uetake H, Sugihara K. Screening for epigenetically masked genes in colorectal cancer Using 5-Aza-2′-deoxycytidine, microarray and gene expression profile. Cancer Genomics Proteomics 2012; 9(2): 67–75
28. Sabates-Bellver J, Van der Flier LG, de Palo M, Cattaneo E, Maake C, Rehrauer H, Laczko E, Kurowski MA, Bujnicki JM, Menigatti M, Luz J, Ranalli TV, Gomes V, Pastorelli A, Faggiani R, Anti M, Jiricny J, Clevers H, Marra G. Transcriptome profile of human colorectal adenomas. Mol Cancer Res 2007; 5(12): 1263–1275
29. Valcz G, Patai AV, Kalmár A, Péterfia B, Fűri I, Wichmann B, Műzes G, Sipos F, Krenács T, Mihály E, Spisák S, Molnár B, Tulassay Z. Myofibroblast-derived SFRP1 as potential inhibitor of colorectal carcinoma field effect. PLoS One 2014; 9(11): e106143
30. Galamb O, Györffy B, Sipos F, Spisák S, Németh AM, Miheller P, Tulassay Z, Dinya E, Molnár B. Inflammation, adenoma and cancer: objective classification of colon biopsy specimens with gene expression signature. Dis Markers 2008; 25(1): 1–16
31. Sun L, Hui AM, Su Q, Vortmeyer A, Kotliarov Y, Pastorino S, Passaniti A, Menon J, Walling J, Bailey R, Rosenblum M, Mikkelsen T, Fine HA. Neuronal and glioma-derived stem cell factor induces angiogenesis within the brain. Cancer Cell 2006; 9 (4): 287–300
32. Demeure MJ, Coan KE, Grant CS, Komorowski RA, Stephan E, Sinari S, Mount D, Bussey KJ. PTTG1 overexpression in adrenocortical cancer is associated with poor survival and represents a potential therapeutic target. Surgery 2013; 154(6): 1405–1416, discussion 1416
33. Giordano TJ, Kuick R, Else T, Gauger PG, Vinco M, Bauersfeld J, Sanders D, Thomas DG, Doherty G, Hammer G. Molecular classification and prognostication of adrenocortical tumors by transcriptome profiling. Clin Cancer Res 2009; 15(2): 668–676
34. Pei H, Li L, Fridley BL, Jenkins GD, Kalari KR, Lingle W, Petersen G, Lou Z, Wang L. FKBP51 affects cancer cell response to chemotherapy by negatively regulating Akt. Cancer Cell 2009; 16 (3): 259–266
35. Du W, Cao Z, Song T, Li Y, Liang Y. A feature selection method based on multiple kernel learning with expression profiles of different types. BioData Min 2017; 10(1): 4
36. Badea L, Herlea V, Dima SO, Dumitrascu T, Popescu I. Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia. Hepatogastroenterology 2008; 55(88): 2016–2027
37. Arredouani MS, Lu B, Bhasin M, Eljanne M, Yue W, Mosquera JM, Bubley GJ, Li V, Rubin MA, Libermann TA, Sanda MG. Identification of the transcription factor single-minded homologue 2 as a potential biomarker and immunotherapy target in prostate cancer. Clin Cancer Res 2009; 15(18): 5794–5802
38. Mortensen MM, Høyer S, Lynnerup AS, Ørntoft TF, Sørensen KD, Borre M, Dyrskjøt L. Expression profiling of prostate cancer tissue delineates genes associated with recurrence after prostatectomy. Sci Rep 2015; 5(1): 16018
39. Bowen NJ, Walker LD, Matyunina LV, Logani S, Totten KA, Benigno BB, McDonald JF. Gene expression profiling supports the hypothesis that human ovarian surface epithelia are multipotent and capable of serving as ovarian cancer initiating cells. BMC Med Genomics 2009; 2(1): 71
40. den Boon JA, Pyeon D, Wang SS, Horswill M, Schiffman M, Sherman M, Zuna RE, Wang Z, Hewitt SM, Pearson R, Schott M, Chung L, He Q, Lambert P, Walker J, Newton MA, Wentzensen N, Ahlquist P. Molecular transitions from papillomavirus infection to cervical precancer and cancer: role of stromal estrogen receptor signaling. Proc Natl Acad Sci U S A 2015; 112(25): E3255–E3264
41. Hu L, Ai J, Long H, Liu W, Wang X, Zuo Y, Li Y, Wu Q, Deng Y. Integrative microRNA and gene profiling data analysis reveals novel biomarkers and mechanisms for lung cancer. Oncotarget 2016; 7(8): 8441–8454
42. Jee BA, Lim H, Kwon SM, Jo Y, Park MC, Lee IJ, Woo HG. Molecular classification of basal cell carcinoma of skin by gene expression profiling. Mol Carcinog 2015; 54(12): 1605–1612
43. Choi YL, Tsukasaki K, O'Neill MC, Yamada Y, Onimaru Y, Matsumoto K, Ohashi J, Yamashita Y, Tsutsumi S, Kaneda R, Takada S, Aburatani H, Kamihira S, Nakamura T, Tomonaga M, Mano H. A genomic analysis of adult T-cell leukemia. Oncogene 2007; 26(8): 1245–1255
44. Payton JE, Grieselhuber NR, Chang LW, Murakami M, Geiss GK, Link DC, Nagarajan R, Watson MA, Ley TJ. High throughput digital quantification of mRNA abundance in primary human acute

myeloid leukemia samples. J Clin Invest 2009; 119(6): 1714–1726

45. Kohlmann A, Kipps TJ, Rassenti LZ, Downing JR, Shurtleff SA, Mills KI, Gilkes AF, Hofmann WK, Basso G, Dell'orto MC, Foà R, Chiaretti S, De Vos J, Rauhut S, Papenhausen PR, Hernández JM, Lumbreras E, Yeoh AE, Koay ES, Li R, Liu WM, Williams PM, Wieczorek L, Haferlach T. An international standardization programme towards the application of gene expression profiling in routine leukaemia diagnostics: the Microarray Innovations in Leukemia Study Prephase. Br J Haematol 2008; 142(5): 802–807
46. Haferlach T, Kohlmann A, Wieczorek L, Basso G, Kronnie GT, Béné MC, De Vos J, Hernández JM, Hofmann WK, Mills KI, Gilkes A, Chiaretti S, Shurtleff SA, Kipps TJ, Rassenti LZ, Yeoh AE, Papenhausen PR, Liu WM, Williams PM, Foà R. Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. J Clin Oncol 2010; 28 (15): 2529–2537
47. Filarsky K, Garding A, Becker N, Wolf C, Zucknick M, Claus R, Weichenhan D, Plass C, Döhner H, Stilgenbauer S, Lichter P, Mertens D. Krüppel-like factor 4 (KLF4) inactivation in chronic lymphocytic leukemia correlates with promoter DNA-methylation and can be reversed by inhibition of NOTCH signaling. Haematologica 2016; 101(6): e249–e253
48. Chu XJ, DePinto W, Bartkovitz D, So SS, Vu BT, Packman K, Lukacs C, Ding Q, Jiang N, Wang K, Goelzer P, Yin X, Smith MA, Higgins BX, Chen Y, Xiang Q, Moliterni J, Kaplan G, Graves B, Lovey A, Fotouhi N. Discovery of [4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](2,3-difluoro-6- methoxyphenyl)methanone (R547), a potent and selective cyclin-dependent kinase inhibitor with significant *in vivo* antitumor activity. J Med Chem 2006; 49(22): 6549–6560
49. Myrthue A, Rademacher BL, Pittsenbarger J, Kutyba-Brooks B, Gantner M, Qian DZ, Beer TM. The iroquois homeobox gene 5 is regulated by 1,25-dihydroxyvitamin D3 in human prostate cancer and regulates apoptosis and the cell cycle in LNCaP prostate cancer cells. Clin Cancer Res 2008; 14(11): 3562–3570
50. Szafron LM, Balcerak A, Grzybowska EA, Pienkowska-Grela B, Podgorska A, Zub R, Olbryt M, Pamula-Pilat J, Lisowska KM, Grzybowska E, Rubel T, Dansonka-Mieszkowska A, Konopka B, Kulesza M, Lukasik M, Kupryjanczyk J. The putative oncogene, CRNDE, is a negative prognostic factor in ovarian cancer patients. Oncotarget 2015; 6(41): 43897–43910
51. Wang Y, Wang Y, Li J, Zhang Y, Yin H, Han B. CRNDE, a long-noncoding RNA, promotes glioma cell growth and invasion through mTOR signaling. Cancer Lett 2015; 367(2): 122–128
52. Zheng J, Liu X, Wang P, Xue Y, Ma J, Qu C, Liu Y. CRNDE promotes malignant progression of glioma by attenuating miR-384/PIWIL4/STAT3 axis. Mol Ther 2016; 24(7): 1199–1215
53. Gao H, Song X, Kang T, Yan B, Feng L, Gao L, Ai L, Liu X, Yu J, Li H. Long noncoding RNA CRNDE functions as a competing endogenous RNA to promote metastasis and oxaliplatin resistance by sponging miR-136 in colorectal cancer. Onco Targets Ther 2017; 10: 205–216
54. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES, Rinn JL. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A 2009; 106(28): 11667–11672
55. Szafron LM, Balcerak A, Grzybowska EA, Pienkowska-Grela B, Felisiak-Golabek A, Podgorska A, Kulesza M, Nowak N, Pomorski P, Wysocki J, Rubel T, Dansonka-Mieszkowska A, Konopka B, Lukasik M, Kupryjanczyk J. The novel gene CRNDE encodes a nuclear peptide (CRNDEP) which is overexpressed in highly proliferating tissues. PLoS One 2015; 10(5): e0127475