High expression of density-regulated re-initiation and release factor drives tumourigenesis and affects clinical outcome

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Abstract. Previously, certain experiments have suggested that density-regulated re-initiation and release factor (*DENR*) could serve important roles in cancer, however, to the best of our knowledge, a comprehensive analysis of *DENR* and its association with cancer patient survival is lacking. The aim of the current study was to investigate the expression of *DENR* in multiple tumour types and to evaluate the effects of *DENR* on survival in malignancies. Sample expression profiles were downloaded from the Gene Expression Omnibus database. Association between *DENR* expression and clinicopathological features was analysed by Chi-square tests. The effects of *DENR* on survival were evaluated by Kaplan-Meier analysis. The results of the current study demonstrate that *DENR* expression was upregulated in nine cancer types. High *DENR* expression indicated poor prognosis of patients. The results of the present study demonstrated that *DENR* is highly expressed in multiple tumour types and may be used as a potential prognostic marker and therapeutic target.

Introduction

The density-regulated re-initiation and release factor (*DENR*) gene has been mapped to the 12q24 region of chromosome 12 in humans (1). *DENR* encodes a 198 amino acid protein. Deyo *et al* (1) identified that *DENR* encodes a novel protein, the expression of which is upregulated in cells cultured at high density, in comparison with those grown at low density. Cell-cell contact is similarly important to immune system function and malignant tumour metastasis (2,3). Certain studies have suggested that alterations in cell density lead to changes in the biology of a cell, including modulated expression of differentiation properties of LLC-PK1 cells (4) and gene transcriptional activity, including *MYC proto-oncogene, BHLH transcription factor* (5). One potential initiating mechanism for *DENR* increase is cell contact itself. Reinert *et al* (6) revealed novel actions of *MCT1*, the monocarboxylate transporter 1 gene located at chromosome Xq22-24 (7), whose protein product is comprised of an SUI1 domain involving the translation initiation factor, eukaryotic initiation factor 2 (eIF2) (8). Reinert *et al* (6) identified that MCT1 is a cap-binding protein that recruits and interacts with DENR and subsequently, modifies the mRNA translational profile. Using a yeast two-hybrid method, MCT1 was demonstrated to interact with DENR involving the SUI1 domain, which may play a role in the pathogenesis of human breast carcinoma (6-9). Skabkin *et al* (10) studied the interactions of Ligatin and MCT-1/DENR and identified related proteins that singly (Ligatin) or jointly (MCT-1 and DENR) elevate efficient eIF2-independent recruitment of Met-tRNA to 40S/mRNA complexes, and demonstrated that MCT-1/DENR could elevate the release of mRNA. A report by Schleich *et al* (11) suggested that DENR affects the translation of mRNAs involved in cell proliferation. Recently, a study identified that *DENR* was highly associated with glioma susceptibility (12). Although, to the best of our knowledge, the specific role of *DENR* has not been elucidated in humans, these studies suggest an important role in cancer. However, a comprehensive analysis of *DENR* expression and its relation to the survival of cancer patients is lacking.

The current study evaluated the expression of *DENR* in normal and cancerous tissues in humans. In addition, the current study analysed the significance of *DENR* in cancer prognosis using publicly available datasets of gene expression coupled with clinical outcomes.

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Materials and methods

Data collection. The expression status of *DENR* in human healthy and tumour tissues was studied using the GEO dataset (http://www.ncbi.nlm.nih.gov/geo/), which included microarray-based experiments measuring mRNA expression. The primary filtering criteria were set to 'Cancer vs. Normal Analysis' and 'Differential Analysis', and the raw data. (CEL format) of each dataset were obtained online. The following dataset were included in the present study: GSE15852 (13); GSE8671 (14); GSE13911 (15); GSE10072 (16); GSE6344 (17); GSE14520 (18); GSE15471 (19); GSE9844 (20) and GSE5563 (21). A standard data normalisation process was used for all datasets. The platform filtering criterion was set to 'Affymetrix U133' to minimise platform variation when detecting differences in *DENR* expression between cancer types. The Affymetrix U133 platform includes three types of arrays: Human Genome U133A 2.0, Human Genome U133A&B and Human Genome U133 Plus 2.0 array. These arrays differed by the number of probe sets on the chip, but all included *DENR*. The association between *DENR* expression and clinicopathological features was analysed, and the relationship between *DENR* and survival was calculated using the GEO dataset and the TCGA (https://cancergenome.nih.gov/). The following clinicopathological features and survival dataset were included in the present study: GSE15459 (22); GSE30219 (23); GSE27020 (24); and breast, kidney, rectal, pancreatic cancer primary data from TCGA (25). Finally, the expression profiles and clinical information for 3,389 tissue samples from nine common cancer types were downloaded, including breast, tongue, gastric, colorectal, lung, renal, pancreatic and vulvar cancers and hepatocellular carcinoma.

Data pre-processing and representation. Samples were defined as either carcinoma or normal. Datasets from the nine cancer types were normalised by the RMA algorithm (26) using R statistical 3.1 software (https://www.r-project.org/). Expression values of *DENR* were dichotomised into low- and high-expression groups using the median as a cut-off value.

Data analysis. Data were analysed using R statistical 3.1 software. Paired or unpaired t-tests were used to analyse expression differences between carcinoma and normal groups. Associations between gene expression and clinicopathological characteristics were evaluated by Chi-square test. Overall survival and recurrence-free survival were evaluated by Kaplan-Meier analysis and a log-rank test. GSEA was used to predict the gene sets modulated by *DENR*. Expression differences were analysed between normal and carcinoma tissues by paired or unpaired t-tests with threshold values of $P<0.05$. $P<0.05$ was considered to indicate a statistically significant difference. Bars indicate the median and the interquartile range of the dataset analysis in the present study.

Results

DENR upregulation in multiple cancers. To examine *DENR* expression in human cancers the tumour expression profiles were downloaded and normalised by RMA. As demonstrated in Fig. 1, *DENR* expression was significantly increased in the following cancer tissues compared with normal tissues: Breast cancer (43 cancer tissues, 43 normal tissues; GSE15852; $P=1.591x10^{-05}$), colorectal cancer (32 cancer tissues, 32 normal tissues; GSE8671; $P=9.991x10^{-13}$), gastric cancer (31 cancer tissues, 31 normal tissues; GSE13911; $P=1.179x10^{-06}$), lung cancer (58 cancer tissues, 49 normal tissues; GSE10072; $P=2.116x10^{-11}$), renal cancer (10 cancer tissues, 10 normal tissues; GSE6344; $P=0.002771$), hepatocellular carcinoma (247 cancer tissues, 239 normal tissues; GSE14520; $P<2.2x10^{-16}$), pancreatic cancer (39 cancer tissues, 39 normal tissues; GSE15471; $P=2.249x10^{-05}$), tongue cancer (26 cancer tissues, 12 normal tissues; GSE9844; $P=0.006865$) and vulvar cancer (9 cancer tissues, 10 normal tissues; GSE5563; $P=3.844x10^{-07}$).

Associations between DENR expression and clinicopathological features of patients with nine common cancer types. The data summarised in Table I reveal significant associations between *DENR* expression and clinicopathological features of patients with multiple cancer types, including hepatocellular carcinoma (primary data from GSE14520), gastric cancer (primary data from GSE15459), lung cancer (primary data from GSE30219), breast cancer (primary data from TCGA), kidney cancer (primary data from TCGA), rectal cancer (primary data from TCGA) and pancreatic cancer (primary data from TCGA). Associations between *DENR* expression and clinicopathological features for head and neck cancer (primary data from GSE9844) including, age, gender and lymph node metastases were not significant. In addition, data involving clinicopathological features for vulvar cancer in public databases were not available.

The results presented in Table I demonstrate that increased *DENR* expression was significantly associated with more aggressive phenotypes. This was measured by the American Joint Committee on Cancer (AJCC) classification of malignant tumours including T, N, M and TNM stages (27). *DENR* expression was significantly higher in advanced AJCC tumour stages than in early tumour stages in multiple cancers including hepatocellular cancer, lung cancer, breast cancer, kidney cancer and rectal cancer. *DENR* expression was significantly elevated in lymph node metastases compared to that in non-lymph node metastases in lung cancer. High *DENR* expression was positively associated with histological tumour subtype and Lauren classification (27) in gastric cancer. *DENR* expression was significantly increased in high α-fetoprotein (AFP) tumours compared with low AFP tumours in hepatocellular cancer.

Association of DENR expression with survival outcome in gastric cancer, hepatocellular carcinoma, lung cancer, kidney cancer and laryngeal cancer. DENR expression (low *DENR*, n=96; high *DENR*, n=96) in gastric cancer tissues (primary data from GSE15459) was significantly associated with reduced overall survival ($\chi^2=5.1$ and $P=0.0235$ in log-rank test; Fig. 2A). Expression of *DENR* (low *DENR*, n=110; high *DENR*, n=111) in hepatocellular carcinoma tissues (primary data from GSE14520) was significantly associated with worse overall survival ($\chi^2=4.6$ and $P=0.0329$ in log-rank test; Fig. 2B). In lung cancer tissues (primary data from GSE30219), *DENR* expression (low *DENR*, n=146; high *DENR*, n=147) was significantly associated with poor overall survival

Table I. Correlation between *DENR* expression and clinicopathological features of patients with nine common cancer types.

Type of cancer	Characteristic	Case (n)	*DENR* expression		χ^2 value	P-value
			High (n)	Low (n)		
Hepatocellular carcinoma (primary data from GSE14520)	AFP (ng/ml^{-1})					
	>300	100	69	31	25.407	<0.001
	≤300	118	41	77		
	AJCC TNM stage					
	I	93	41	52	6.005	0.049
	II	77	37	40		
	III	39	32	17		
	CLIP stage					
	0	97	33	64	18.308	<0.001
	1	74	47	27		
	2-5	48	30	18		
Gastric cancer (primary data from GSE15459)	Lauren classification					
	Diffuse	75	29	46	6.348	0.041
	Mixed	99	57	42		
	Intestinal	18	10	8		
	Subtype					
	Invasive	51	28	23	55.099	<0.001
	Metabolic	40	2	28		
	Unstable	70	54	16		
	Proliferative	31	12	19		
Lung cancer (primary data from GSE30219)	AJCC T stage					
	I	166	68	98	11.694	0.008
	II	69	42	27		
	III	31	20	11		
	IV	21	12	9		
	Lymph node metastasis					
	Positive	93	63	30	16.878	<0.001
	Negative	208	83	115		
Breast invasive carcinoma (primary data from TCGA)	Age/year					
	≤65	759	357	402	8.461	0.004
	>65	319	181	138		
	AJCC T stage					
	T1	279	126	153	13.487	0.004
	T2	633	340	293		
	T3	138	56	82		
	T4	40	25	15		
	AJCC N stage					
	N0	515	253	262	9.324	0.025
	N1	361	173	188		
	N2	120	75	45		
	N3	77	34	43		
Kidney chromophobe carcinoma (primary data from TCGA)	AJCC TNM stage					
	I-III	60	27	33	6.600	0.010
	IV	6	6	0		
Rectal adenocarcinoma (primary data from TCGA)	AJCC T stage					
	T1	4	3	1	9.423	0.024
	T2	13	9	4		
	T3	65	34	31		
	T4	10	1	9		

Table I. Continued.

Type of cancer	Characteristic	Case (n)	*DENR* expression		χ^2 value	P-value
			High (n)	Low (n)		
Pancreatic adenocarcinoma (primary data from TCGA)	Sex					
	Male	98	60	38	10.989	<0.001
	Female	80	29	51		
Head and neck carcinoma and vulvar carcinoma	Clinicopathological feature data for head and neck cancer and vulvar cancer in public databases were either non-significant or not available.					

Data analysed by Chi-square test. *DENR*, density-regulated re-initiation and release factor; AFP, α-fetoprotein; AJCC, American Joint Committee on Cancer; CLIP, Cancer of the Liver Italian Program; TNM, tumour-node-metastases; T, tumour; N, lymph node; n, number of samples; TCGA, The Cancer Genome Atlas.

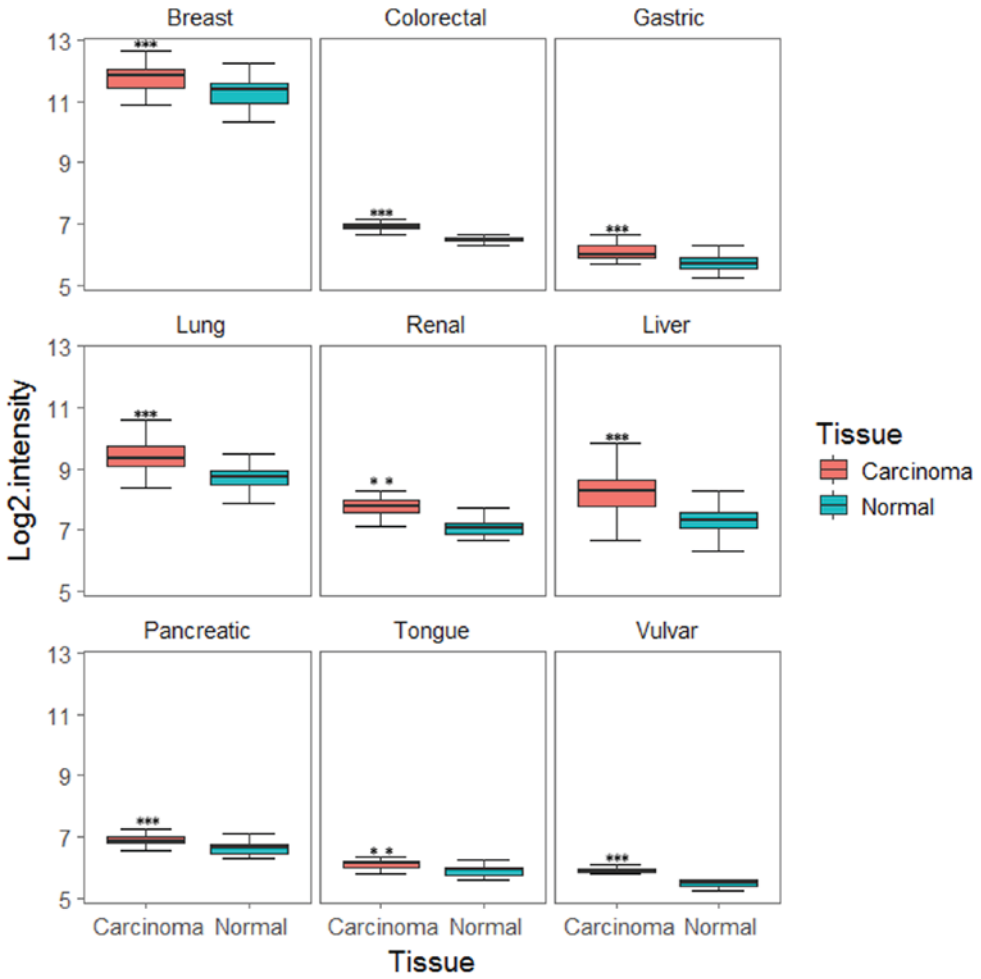

Figure 1. Expression of *DENR* in multiple cancer types. These data were extracted from the Gene Expression Omnibus database. *DENR* was upregulated in breast, gastric, colorectal, renal, lung, liver, pancreatic, tongue and vulvar cancers. **P<0.01, ***P<0.001 vs. normal, by paired or unpaired t-test. *DENR*, density-regulated re-initiation and release factor.

(χ^2=22.3 and P=2.28x10^{-06} in log-rank test; Fig. 2C). For lung cancer (primary data from GSE30219), expression of *DENR* (low *DENR*, n=146; high *DENR*, n=147) was significantly associated with diminished recurrence-free survival (χ^2=17.1 and P=3.6x10^{-05} in log-rank test; Fig. 2D). *DENR* expression (low *DENR*, n=33; high *DENR*, n=33) in kidney cancer tissues (primary data from TCGA) was significantly associated with poor overall survival (χ^2=4.4 and P=0.0351 in log-rank test; Fig. 2E). *DENR* levels (low *DENR*, n=53; high *DENR*, n=54) in laryngeal cancer tissues (primary data from GSE27020) were significantly associated with reduced recurrence-free survival (χ^2=4.8 and P=0.0284 in log-rank test; Fig. 2F).

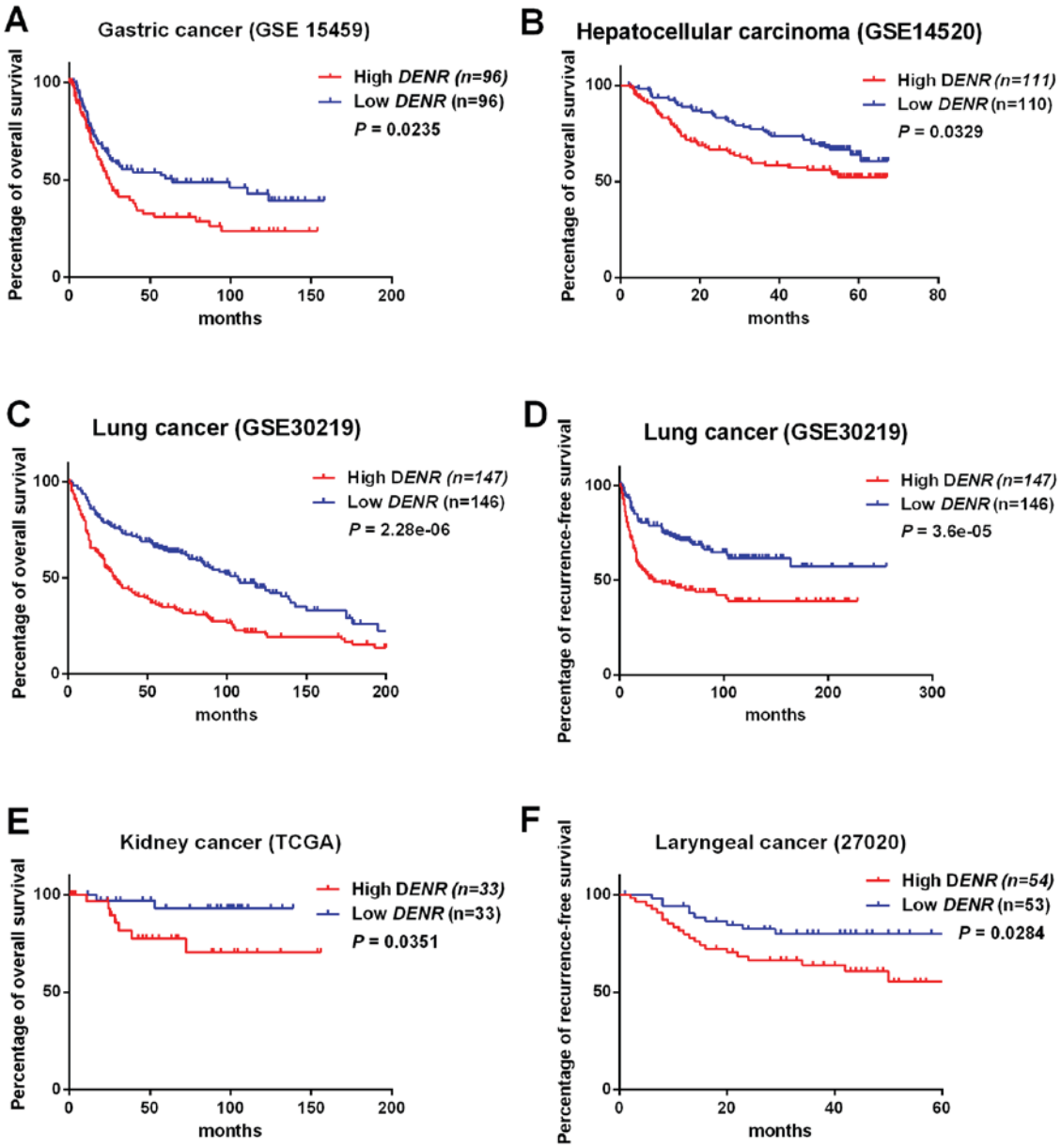

Figure 2. Association of *DENR* expression with patient prognosis in gastric cancer, hepatocellular carcinoma, lung cancer, kidney cancer and laryngeal cancer. The association was investigated by Kaplan-Meier analysis and log-rank test. Overall survival of (A) patients with gastric cancer (data from GSE15459) and (B) patients with hepatocellular carcinoma (data from GSE14520). (C) Overall survival and (D) recurrence-free survival of patients with lung cancer (data from GSE 30219). (E) Overall survival of patients with kidney cancer (data from TCGA) and (F) recurrence-free survival of patients with laryngeal cancer (data from GSE27020). *DENR*, density-regulated re-initiation and release factor; GSE, gene set enrichment.

In summary, these results demonstrate that high *DENR* expression is indicative of a poor prognosis for patients with hepatocellular carcinoma, gastric cancer, kidney cancer, laryngeal cancer and lung cancer.

GSEA for lung cancer tissues with high DENR expression. Fig. 3 demonstrates the results of GSEA for lung cancer tissues with high *DENR* expression (primary data from GSE30219). The curated gene set was used as an example dataset. The results revealed that enriched gene sets were associated with CELL_CYCLE [P<0.001, false discovery rate (FDR)=0.068, enrichment score (ES)=0.793; Fig. 3A], CR_REPAIR (cancer-related DNA repair) (P=0.012, FDR=0.071, ES=0.670; Fig. 3B), DNA_DAMAGE_SIGNALLING (P=0.002, FDR=0.056, ES=0.558; Fig. 3C) and MRNA_SPLICING (P<0.001, FDR=0.003, ES=0.644; Fig. 3D). These findings indicated that *DENR* regulates cell cycle, cancer-related DNA repair, DNA damage signalling and mRNA splicing activities.

Discussion

In the current study, it has been demonstrated that *DENR* expression is upregulated in different cancer tissues compared with that observed in normal tissues. Deyo *et al* (1) demonstrated that the *DENR* gene maps to human chromosome 12. Skabkin *et al* (28) revealed that the release of tRNA and mRNA is associated with eukaryotic translation initiation factor 1 (eIF1)/eIF1A, Ligatin or MCT-1/*DENR*. Furthermore,

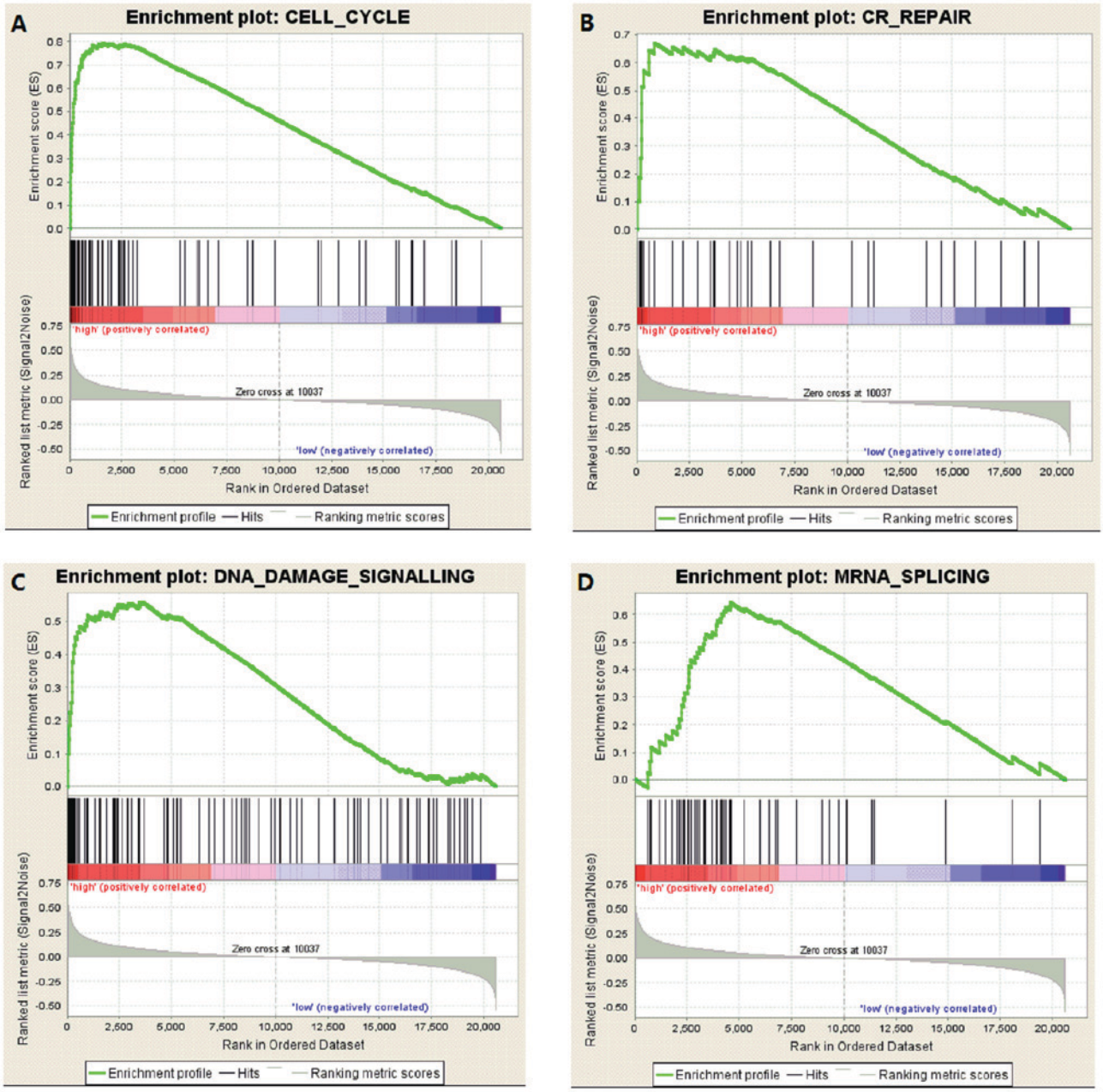

Figure 3. GSEA for lung cancer tissues with high *DENR* expression (data from GSE30219). GSEA revealed that the enriched gene sets were associated with (A) cell cycle, (B) CR_REPAIR (cancer DNA related repair), (C) DNA damage signalling and (D) mRNA splicing. GSEA, gene set enrichment analysis; *DENR*, density-regulated re-initiation and release factor.

Schleich *et al* (11) demonstrated that the regulation of the translation of a specific set of mRNAs is associated with *DENR*. The results of GSEA for lung cancer tissues with high *DENR* expression identified in the current study established that enriched gene sets were associated with the MRNA_SPLICING categorisation. These findings indicated that *DENR* regulates mRNA splicing. A previous study disclosed that cells harbour a previously unappreciated translational control system that involves *DENR* and exerts a key role in tissue development and supporting cellular proliferation (11). The current study demonstrates that *DENR* is associated with cell cycle, CR_REPAIR (cancer-related DNA repair), DNA damage signalling, disrupted DNA replication, DNA repair and cell cycle progression, which promotes uncontrolled proliferation of tumour cells (29). Similarly, the current study identified that elevated *DENR* expression was significantly associated with advanced AJCC tumour stages in multiple cancer types including hepatocellular cancer, lung cancer, breast cancer, kidney cancer and rectal cancer.

Advanced tumour stage, tumour size and lymph node metastasis are each indicative of poor prognosis (30,31). High *DENR* expression was associated with these disadvantageous features in the current study and was also associated with poor overall survival or recurrence-free survival in hepatocellular carcinoma, gastric cancer, kidney cancer, laryngeal cancer and lung cancer. In hepatocellular carcinoma, *DENR* expression was significantly increased in high AFP tumours compared with low AFP tumours. A recent study suggested that AFP is

associated with malignant hepatic tumours (32). The current study revealed high *DENR* expression was positively associated with the histological tumour invasive subtype and metabolic subtype in gastric cancer. A previous study demonstrated that invasive subtype is associated with malignant gastric cancer and poor prognosis (33). The current study revealed that *DENR* expression in gastric cancer tissues is significantly associated with overall survival. High *DENR* expression is indicative of poor prognosis for patients with gastric cancer.

In conclusion, the current study demonstrated that *DENR* is highly expressed in multiple cancer types and is associated with poor survival of patients with hepatocellular carcinoma, gastric cancer, kidney cancer, laryngeal cancer and lung cancer. Elevated expression of *DENR* may contribute to tumourigenesis and affect clinical outcome.

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Availability of data and materials

The results of the present study are in whole or part based upon data generated by the TCGA Research Network (http://cancergenome.nih.gov/) and the GEO dataset (http://www.ncbi.nlm.nih.gov/geo/).

Authors' contributions

DW and SZ conceived and designed the study. Data were curated and analyzed by DW, SZ, LW and PZ. Data collection and software analysis were performed by DW, LW, CR and MW. All authors contributed to the writing of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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