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

DAZHI WANG1-3, LANYING WANG2, CHUNLING REN4, PEI ZHANG5, MENGMENG WANG6 and SHUYU ZHANG2

1Oncology Center of Qingdao Municipal Hospital; 2Pharmacy Department of Qingdao Municipal Hospital, School of Medicine, Qingdao University, Qingdao, Shandong 266071; 3Department of Pharmacy, Cheeloo College of Medicine, Shandong University, Jinan, Shandong 250100; 4Pharmacy Department of Qingdao Women and Children’s Hospital, School of Medicine, Qingdao University, Qingdao, Shandong 266071; 5Pharmacy Department of The Fifth Affiliated Hospital, Xinjiang Medical University, Urumqi, Xinjiang 830011; 6Pharmacy Department of Baoding No. 2 Center Hospital, Baoding, Hebei 072750, P.R. China

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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 clinico-pathological 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 SU11 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 SU11 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.
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^-46), colorectal cancer (32 cancer tissues, 32 normal tissues; GSE8671; P=9.991x10^-4), 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^-16), renal cancer (10 cancer tissues, 10 normal tissues; GSE6344; P=0.002771), hepatocellular carcinoma (247 cancer tissues, 239 normal tissues; GSE14520; P=0.02x10^-06), 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 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 (χ^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 (χ^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.
| Type of cancer (primary data)                                      | Characteristic                  | Case (n) | High (n) | Low (n) | $\chi^2$ value | P-value |
|--------------------------------------------------------------------|---------------------------------|----------|----------|---------|----------------|---------|
| **Hepatocellular carcinoma**                                        | **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-Ill                           | 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) | High (n) | Low (n) | $\chi^2$ value | P-value |
|-----------------------------------------------|----------------|----------|----------|---------|----------------|---------|
| 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.

($\chi^2$=22.3 and P=2.28x10^-6 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 ($\chi^2$=17.1 and P=3.6x10^-6 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 reduced recurrence-free survival ($\chi^2$=4.8 and P=0.0284 in log-rank test; Fig. 2F).
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, 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,
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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