# DDR1 promoting mesenchymal to epithelial transition in Diffuse B-Cell Lymphoma

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***Abstract***

*Diffuse large B-cell lymphoma (DLBC) is the most common subtype of non-Hodgkin lymphoma (NHL), presenting significant challenges due to its aggressiveness and frequent relapses. It arises from malignant B-lymphocytes, affecting lymph nodes and extra-nodal sites like the central nervous system, bone marrow and gastrointestinal tract. In India, DLBC contributes to 9,000-14,000 of the 30,000-35,000 annual NHL cases, making it a substantial health burden. This study aims to investigate the role of DDR1 (Discoidin Domain Receptor 1-receptor tyrosine kinase) in DLBC, its correlation with epithelial and mesenchymal markers and its association with tumor progression and patient survival. DDR1 was found to be 3.5-fold upregulated in DLBC as compared to normal.*

*It positively correlates with mesenchymal markers like VIM, ZEB2 and PXN, indicating a role in mesenchymal-to-epithelial transition (MET). Conversely, DDR1 negatively correlates with epithelial markers such as CDH1, KRT7, COL1A1, CLDN3 and CLDN1. Stage plot analysis showed reduced DDR1 expression in stage IV of DLBC, aligning with tumor metastasis and secondary tumor growth. Survival analysis revealed that high DDR1 levels are associated with poor prognosis. These findings suggest DDR1 as a key biomarker influencing DLBC progression, metastasis and survival.*

**Keywords:** MET, DLBC, DDR1, Metastasis, GBC, ABC.

## Introduction

The most prevalent subtype of non-Hodgkin lymphoma is diffuse large B-cell lymphoma (DLBC), which poses a serious treatment challenge because of its aggressiveness and variable features. It is most common in older adults with a median age of 70 at diagnosis, while it can also strike younger people and in rare cases, children. A minor male predominance is observed in the disease[21]. It is mainly characterized by the malignant growth of B-lymphocytes, which can occur in extra nodal locations such as the central nervous system, bone marrow, gastrointestinal tract and other organs, or in lymph nodes. Rapidly growing lymph nodes, fever, sweats at night and weight loss are common signs of DLBC. Extra nodal mass lesions can also occur in certain situations. Based on molecular, genetic and clinical characteristics, DLBC is a diverse illness with multiple subgroups[2].

Systemic symptoms such as fever, sweats at night and unexplained weight loss, are frequently seen and may indicate severe illness[33]. The two main subtypes, Germinal Centre B-cell-like (GCB) and Activated B-cell-like (ABC), are determined by cell-of-origin (COO) categorisation utilising immunohistochemical markers or gene expression analysis. Germinal centre B-cell-derived (GCB) DLBC often has a good prognosis while post-germinal centre Activated B-cell-derived (ABC) DLBC is linked to worse results. Due to their resistance to conventional chemotherapy, some aggressive subtypes, like double-hit and triple-hit lymphomas, which are characterised by chromosomal rearrangements affecting the MYC and BCL2 genes, have an exceptionally dismal prognosis[33].

DLBC accounts for approximately 30-40% of all NHL cases worldwide. In India, an estimated 30,000–35,000 new cases of NHL per year is reported, of which DLBC accounts for roughly 9,000–14,000 cases, as it represents a substantial health burden. Among all lymphomas, including Hodgkin and non-Hodgkin forms, 25-30% are of DLBC[27,32]. Geographically, the prevalence of DLBCL varies due to viral, environmental and genetic variables. Aggressive DLBC subtypes frequently overexpressed FOXM1, a transcription factor associated with cell survival and proliferation. Tumor necrosis factor receptor family member, CD40 is linked to B-cell activation and survival and is involved in immunological control[25].

DDR1 is overexpressed in some DLBC subtypes, especially the ABC subtype, where it improves survival signalling and modifies the tumour microenvironment, hence promoting tumour growth. DDR1 expression is linked to the activation of downstream pathways that support cell invasion, proliferation and apoptosis resistance including PI3K/AKT and MAPK/ERK[44]. Rituximab, a monoclonal antibody that targets CD20, is used in conjunction with the chemotherapeutic drugs cyclophosphamide, doxorubicin, vincristine and prednisone as part of the R-CHOP regimen, which is the standard treatment for DLBCL. For 60–70% of patients, this regimen results in remission; nonetheless, refractory or relapsed cases continue to be a major concern[8].

A biological process known as the mesenchymal-to-epithelial transition (MET) occurs when cells change from a mesenchymal state, which is characterised by invasiveness and motility to an epithelial state which is characterised by polarity and cell adhesion (Figure 1). The more widely

discussed epithelial-to-mesenchymal transition (EMT) is the opposite of this process. Both MET and EMT are essential for a number of physiological and pathological processes, such as tissue regeneration, cancer progression and embryonic development[19]. In organogenesis, MET is essential during embryonic development. For example, mesenchymal cells go through MET during kidney development to create the nephron's epithelial structures[39].

MET is linked to the later phases of metastasis in the context of cancer. At distant locations, tumour cells that have undergone EMT to spread from the original tumour may MET-revert to an epithelial phenotype[19]. By allowing the cancer cells to re-adopt epithelial traits including cell-cell adhesion and contact with the surrounding milieu, this reversion promotes the formation and colonisation of subsequent tumours (Figure 1). Cancer cells can take advantage of cellular plasticity through the dynamic interaction between EMT and MET, which increases their capacity for metastasis and adaptability in a variety of tissue settings[39].

A key mechanism that supports the adaptability of cellular phenotypes in both healthy development and the advancement of malignancy is MET. MET is necessary for organ development and tissue regeneration in normal physiology because it promotes the development of epithelial tissues from mesenchymal progenitors. In tumour biology, MET plays a key part in the metastatic cascade by allowing dispersed cancer cells to form additional tumours by returning to an epithelial state. Knowing the regulatory processes that control MET may help to develop treatment plans that modify cellular plasticity in the fight against cancer[45].

Discoidin domain receptor 1 (DDR1) belongs to the discoidin domain receptor family, which also comprises of RTKs (Receptor tyrosine kinases) that mostly work with collagen (Figure 2). A key player in many cellular functions including adhesion, migration, proliferation and differentiation, DDR1 is also linked to carcinogenesis and to the development of several malignancies[16]. Structurally, DDR1 comprises of an extracellular domain region that contains collagen types I through VI, specifically bound by the discoidin domains (Figure 2). The binding takes place at a particular amino acid sequence motif (GVMGFO)[16].

The transmembrane region helps the extracellular environment and intracellular signalling pathways communicating by anchoring the receptor in the cell membrane1. DDR1 is auto-phosphorylated by the intracellular tyrosine kinase when a ligand binds to it, initiating subsequent signalling pathways that are involved in cellular reactions4. DDR1 is a member of the discoidin domain receptors (DDRs) subfamily of RTKs, which also contains DDR2. Although the two receptors' structures are similar, their functions and ligand interactions are different. DDR2 has unique binding properties and functions, whereas DDR1 mostly binds to collagen[29].

DDR1 controls adhesion, migration and proliferation and is widely expressed in epithelial tissues. DDR1 auto-phosphorylates when it binds to collagen, starting downstream signalling cascades like the PI3K/AKT, MAPK and NF-κB pathways that mediate its effects on matrix remodelling, cell survival and differentiation[14,34]. Specific mutations in DDR1 were found in early research, especially in samples of lung cancer. Subsequent analyses have had difficulty in reproducing these results. For example, despite earlier reports of mutations like R824W in the NCI-H1770 lung cancer cell line, a thorough expression and mutation investigation in lung tumours did not uncover any new mutations in DDR1 or DDR2.

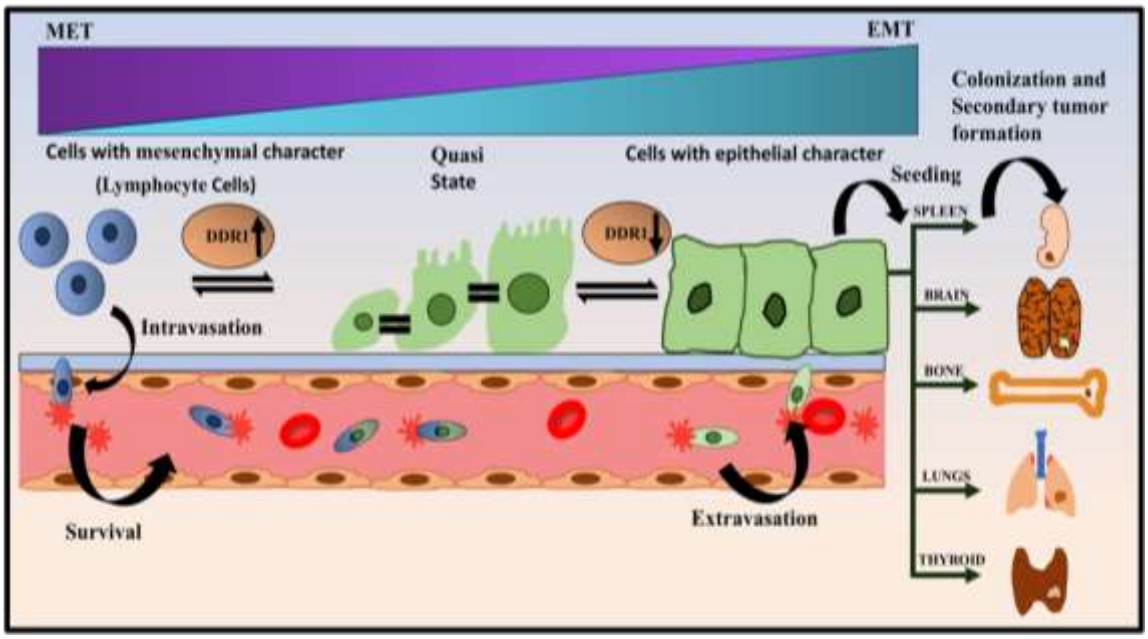


**Figure 1: Graphical Abstract of the manuscript**

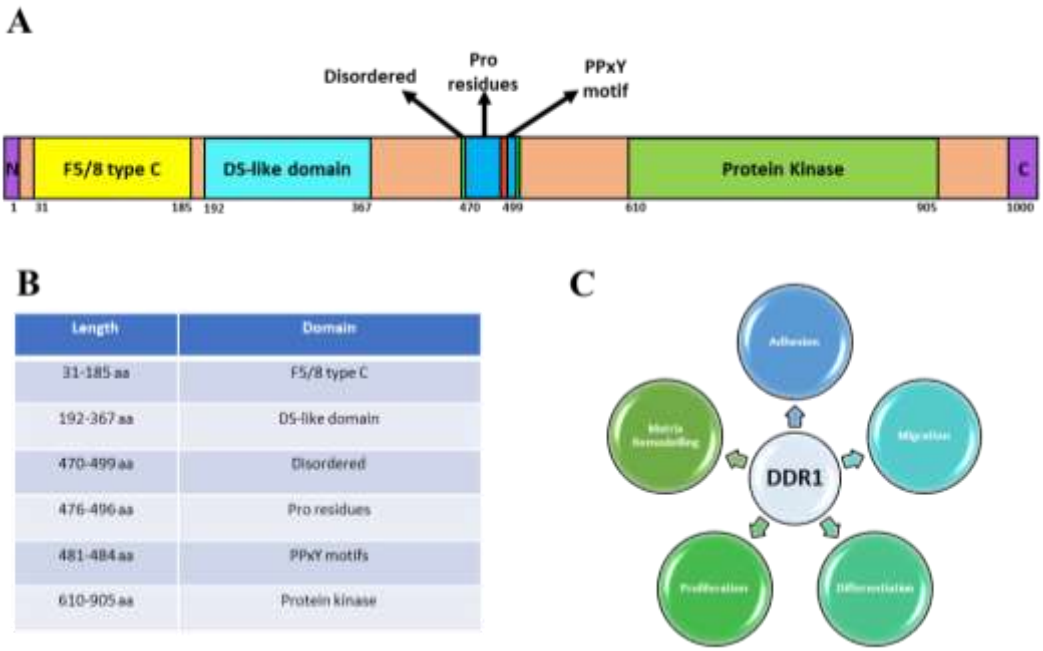


**Figure 2: (A) Domains of DDR1. (B) Descriptive length and domains of DDR1 (C) Function of DDR1**

This implies that DDR1 mutations might be uncommon or restricted to particular populations[13,20]. If mutations are found, they may change DDR1's function and affect how it contributes to carcinogenesis. Constitutive activation or loss of function can result from mutations that impact downstream signalling pathways that control cell motility, proliferation and survival. Mutations could improve the receptor's capacity to stimulate pro-inflammatory reactions or extracellular matrix remodelling, for instance, both of which are essential for the growth of tumours[3,46].

Numerous tumours have been connected to DDR1 expression levels and in certain situations, high expression is associated with a poor prognosis. Due to the small number of known mutations, it is yet unknown how certain cancer types are related to DDR1 mutations. Tumorigenesis in some malignancies, like breast and lung cancer, may be primarily driven by changed expression rather than mutation[17].

Studies reveal that DDR1 is often expressed in high-grade brain tumours, indicating that it has a selective role in tumour cells as opposed to healthy brain tissue[43]. Elevated DDR1 levels have been correlated with increased tumor size, indicating its potential as a biomarker for tumor progression[12]. DDR1 expression is also considerably greater in brain tumour tissues and serum samples, according to a study, which supports its applicability in the setting of brain cancers[11]. High DDR1 expression has been associated with increased recurrence and metastasis, lower overall patient survival rates and poor response to neoadjuvant treatment in osteosarcoma, the most prevalent type of bone cancer.

Research using DDR1-specific inhibitors showed that osteosarcoma cell growth and proliferation were considerably decreased when DDR1 expression was knocked down, indicating that it may be a promising therapeutic target. The link between DDR1 levels and clinical outcomes underscores its role as an independent predictor of poor prognosis[41]. It has been demonstrated that DDR1 encourages tumour invasion and metastasis in a variety of cancer types. Because it contributes to the malignant behaviour of tumours, its expression is frequently associated with a poor prognosis. For instance, studies indicate that elevated DDR1 levels are associated with increased lymph node invasion and higher-grade tumors in colorectal cancer (CRC)[10].

DDR1 is still a viable target for therapeutic intervention because of its involvement in the development of cancer and the scant evidence of widespread alterations. Targeting DDR1 signalling pathways may increase immune responses against tumours or make tumours more sensitive to current treatments[3]. Numerous diseases such as cancer, fibrosis and cardiovascular disorders, are influenced by mutations or dysregulations in DDR1. For example, DDR1 mutations or overexpression have been linked to DLBC, a disease in which altered matrix connections and signalling dynamics promote tumour growth and immune evasion (Figure 1). The importance of DDR1 to oncogenesis is supported in this context by its function in promoting tumor-ECM communication and preserving the integrity of the extracellular matrix (ECM)[18].

DDR1 interacts with immune cells and other elements to affect the tumour microenvironment. High DDR1 levels have been shown to form a barrier that prevents immune cells from infiltrating tumours, hence accelerating tumour growth and metastasis[15]. Numerous immune cell types, particularly CD8+ T cells and macrophages, have been shown to be negatively correlated with DDR1 expression in

tumours. A poor prognosis is frequently linked to high DDR1 levels because they decrease the number of immune cells in the TME (Tumor microenvironment)[23] (Figure 1). DDR1 has the ability to control the release of cytokines and pro-inflammatory mediators that impact immune responses. For instance, it has been linked to increasing the production of interleukin-6, which influences immune cell behaviour and can encourage tumour growth[46].

DDR1 is a crucial receptor tyrosine kinase that is implicated in a number of biological processes including differentiation, migration and cell adhesion. Its significance in both healthy and diseased physiological processes is highlighted by its interactions with collagen and control of signalling pathways. Its functions in health and illness can be better understood by knowing its ontology which also offers suggestions for possible treatment approaches. DDR1 also stimulates epithelial-mesenchymal transition (EMT), which gives epithelial cells mesenchymal characteristics and increases their capacity for invasion and migration. Increased expression of mesenchymal markers (such vimentin and N-cadherin) and decreased expression of epithelial markers (like E-cadherin) are characteristics of this transition[15].

DDR1 knockdown dramatically reduced the development of tumour spheres *in vitro* and the spread of breast cancer to other organs. Pancreatic cancer cell migration was also suppressed by RNAi-mediated DDR knockdown. Furthermore, the development of Kras-driven lung tumours was considerably reduced in DDR1 deletion mice. Thus, DDR1 targeting may be a promising treatment strategy for aggressive metastatic cancer[15].

In this study, we have analysed that when DLBC reaches the fourth stage of cancer progression, the level of DDR1 drops which could favour the mesenchymal to epithelial transition and ultimately may lead to the formation of secondary tumours. Furthermore, our analysis suggests that regardless of patient demographics or numbers, patients with more body weight are associated with poor survival. Using DLBC-known biomarkers such as CD40 and FOXM1, mesenchymal biomarkers such as VIM, ITGB1, ITGB3, PXN and ZEB2 and epithelial biomarkers such as CDH1, COL1A1 and KRT7, we aim to perform a comprehensive comparative *in silico* study of DDR1.

## Material and Methods

**Expression Profile of DDR1:** We acquired quantitative expression information from The Cancer Genome Atlas (TCGA) project for 23 DLBC patients (https://www.cancer.gov/tcga) and 23 normal tissue samples as a control from the Genotype-Tissue Expression (GTEx) project (https://www.gtexportal.org/home/)[40]. In order to fully examine the way in which our gene of interest is expressed in DLBC, the expression data was quantified using both raw read counts reported in transcripts per million (TPM) in log scale.

**Correlation of DDR1 with other known biomarkers and pathway related genes:** Spearman correlation approach was employed to determine the link between DDR1 expression level and other known biomarkers specific to DLBC and additional genes engaged in molecular cell signalling the pathway involved in the advancement of DLBC, GEPIA2 was used for the correlation analysis[37,38].

**Determining the promoter methylation status in DLBC:** Using UALCAN portal (The University of Alabama at Birmingham CANcer data analysis Portal), the methylation status of the DDR1 promoter was examined for DLBC[5,6]. Datasets from the TCGA database were extracted by the portal and were used for the analysis of promoter methylation.

**Assessment of Direct and Indirect Interaction partners of DDR1:** To determine the functional links between specific genes that interact with MET biomarkers, their co-expression, co-occurrence, direct and indirect interactions, the search tool for the retrieval of interacting genes/proteins database (STRING, version 11.0) was used. The STRING library establishes direct (physical) and indirect (functional) connections between genes based on expected product interactions. Interactions were found based on the genetic environment, experimental data, book references and conversations.

## Results and Discussion

**Expression profile of DDR1 in DLBC:** According to previous reports, DLBC expresses DDR1 at comparatively low levels when compared to other malignancies[46], but according to our study, there is high expression of DDR1 in DLBC which is very significant as shown in figure 3A (Fold change of 3.5 as compared to normal lymphocytes); suggesting a unique role or regulation in this cancer type. Surprisingly the level of DDR1 falls in the fourth stage of DLBC (Figure 3B) which could be co-related with the transition of lymphocytes with mesenchymal character to the epithelial like cells, which could be seeded in other tissues and colonize to form secondary tumor[31].

There has been investigation into the connection between DDR1 expression and patient viability. Elevated DDR1 levels could affect survival results[22]. DDR1 is being studied as a therapeutic target because of its function in regulating immune cell infiltration and its link to treatment resistance. In tumours where it is overexpressed, its regulation may improve the effectiveness of immunotherapies[46]. It has been demonstrated that DDR1 inhibits immune cell infiltration in tumours. Targeting DDR1 may improve T-cell recruitment to the tumour microenvironment, increasing the effectiveness of immune checkpoint inhibitors (ICIs)[42]. In DLBC, aggressive tumour features have been linked to high DDR1 expression. It is specifically hypothesised that DDR1 may promote invasion and metastasis, which can worsen patient outcomes and can accelerate tumour growth[26]. Datasets from TGCA for various carcinoma samples and

GTex for normal sample expression profiles were used to perform the expression profile of DDR1. There is a clear comparative expression with normal as seen in the box plot shown in figure 3B. The expression levels of DDR1 at various DLBC stages are shown in figure 3C. In contrast to stage 3, stage 4 exhibits lower expression and increased variability that could trigger mesenchymal to epithelial transition (MET). Lower expression of DDR1 at this stage is indicated by the median value, which is shown by the bold horizontal line inside the box. The interquartile range (IQR) is represented by the box and the spread of the box indicates variation in DDR1 expression levels for stage 4 samples, indicating that the cancer is progressing towards metastasis.

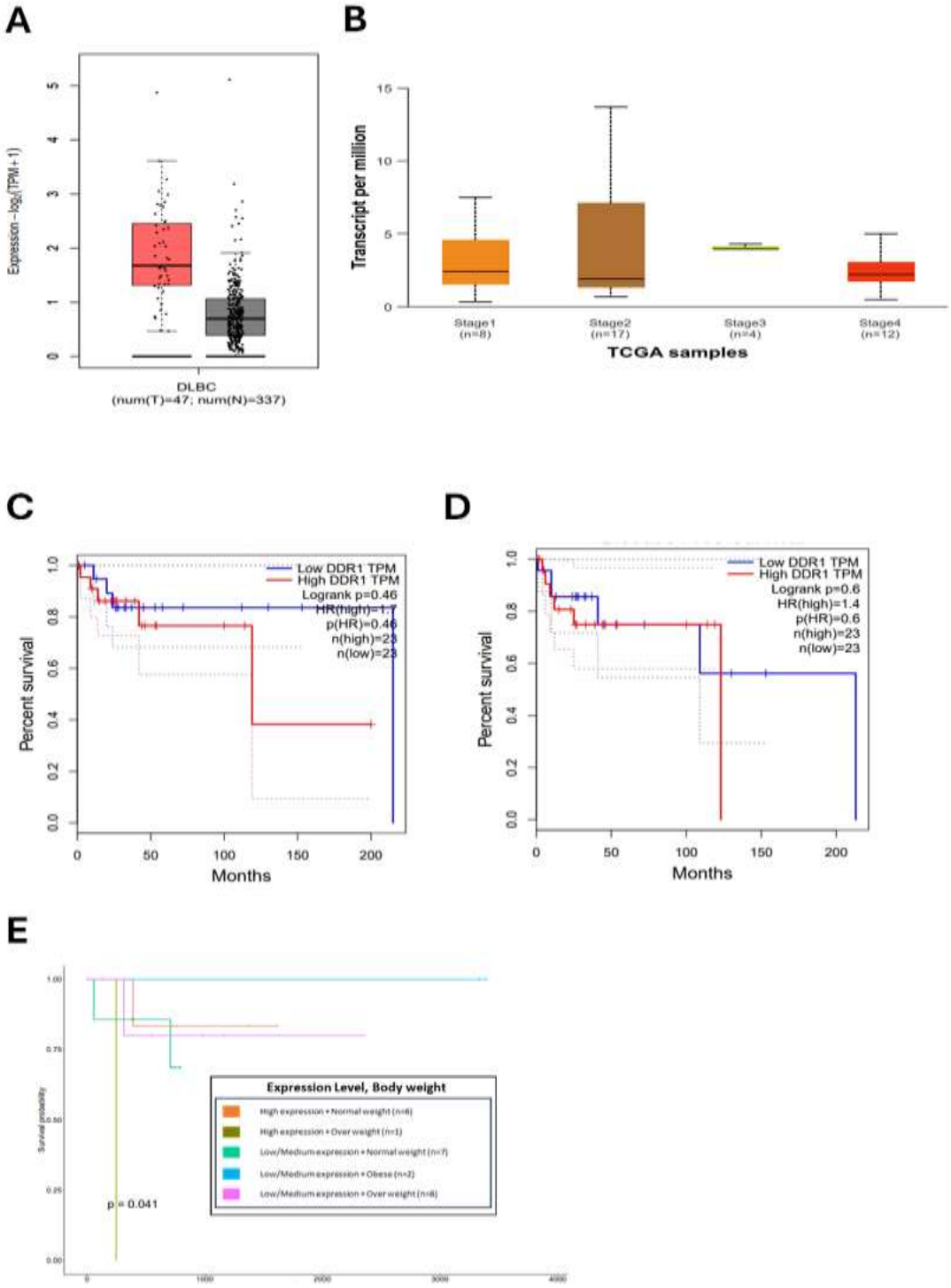


**Figure 3: Expression profile of DDR1 and associated survival (A) Boxplot representing fold change expression analysis of DDR1 in DLBC (Fold Change calculated by dividing with normal expression level) (B) Stageplot-DLBC stage specific expression analysis of DDR1. (C) Kaplan-Meier Plot representing Overall Survival. (D) Kaplan-Meier Plot representing Disease Free Survival. hazard ratios (HR) in log10 depicts associated risk of survival. (E) Kaplan-Meier Plot representing the impact of Body weight and expression of DDR1 on survival of DLBC patients.**

The minimum and highest observed values are represented by the whiskers that extend above and below the box. DDR1 expression lowers as stage 4 of carcinogenesis approaches and this down expression causes the mesenchymal-epithelial transition (MET) to create secondary tumours. Through MET, mesenchymal cells lose their invasive and migrating characteristics and acquire the capacity to form adhere junctions, tight junctions and desmosomes similar to epithelial cells, which allow them to colonise secondary sites and impact the development of metastatic tumours[9].

Figures 3C, D and E depict Kaplan-Meier survival analyses for a study that looked into how DDR1 expression levels affected the survival of patients with diffuse large B-cell lymphoma (DLBC). Overall survival, as shown in figure 3D, is the time between the beginning of observation (such as a diagnosis or course of therapy) and patients with low DDR1 expression (blue line) and those with high DDR1 expression (red line) are the two groups that are being compared in the graph. The survival curves indicate the chances of surviving of a particular group over a period of time in comparative manner, with overlapping curves.

Figure 3D illustrates disease-free survival (DFS), which is the interval between therapy or remission and the onset or advancement of the disease. Similar comparisons between patients with low (blue) and high (red) DDR1 expression are shown in the graph. The two groups' survival curves over time reveal very little variation. Since the log-rank p-value is 0.6, the groups' DFSs do not differ significantly. With a hazard ratio (HR) of 1.4, the high DDR1 group may be at somewhat higher risk of disease recurrence, although this is not statistically significant. The graphs show that in patients with DLBC, DDR1 expression (high vs. low) had no discernible effect on overall survival or disease-free survival but have an impact on those who have a low survival rate. Poor survival results from the high expression of DDR1 have no discernible impact on the survival rate.

The Kaplan-Meier survival curve, depicted in figure 3E, illustrates how body weight and DDR1 expression levels affect the survival of patients with DLBC. The groups' differences in survival are statistically significant, according to the p-value ($p = 0.041$). This implies that the combination of body weight and DDR1 expression level may affect survival outcomes. Over time, the survival probabilities of groups with varied body weight categories and DDR1 expression levels vary. Obesity (brown line) and low DDR1 expression are two categories that appear to have lower survival rates than others. Thus, over-weight is associated with poor survival.

**Methylation Profile of the promoter of DDR1:** Promoter methylation levels of DDR1 in patients with diffuse large B-cell lymphoma (DLBC) among various racial groups were determined. The boxplot was prepared between the two racial group [Asian (n=18) and Caucasian (n=29)] and DNA methylation levels are measured by the beta value, which is shown on the Y-axis. The range of the beta value is 0 (unmethylation) to 1 (hypermethylation). These numbers represent the degree of DDR1 gene promoter methylation. The interquartile range (IQR), which represents the middle 50% of the data, is displayed in the box. The median methylation level is shown by the horizontal line inside the box.

When comparing the Asian and Caucasian groups, the Asian group shows low methylation of DDR1 promoter which may lead to the higher expression of DDR1 protein, further leading to progression of DLBC in Asian population. Variations in promoter methylation levels may affect the expression of the DDR1 gene, which may affect how the gene functions in the onset or progression of DLBC. Disparities in methylation patterns between races may be due to environmental, genetic, or epigenetic reasons. To verify and to investigate the possible influence of these variations on clinical results, more research with bigger sample numbers is needed to be in this direction, but these 18 samples reveal comparatively less methylation in Asian population, which is associated with poor survival (Figure 3C and D).

**Correlation with known biomarkers:** We used the Spearman correlation test to examine the association between the expression levels of the putative biomarker (DDR1) and the established biomarkers (MAP2K1 and CD40). The range of values for the coefficient of correlation (R) is +1 to 1, additionally, it receives 0 if there is no association. Positive correlation might be a sign of co-expression or stimulation, while negative correlation can be a component of inhibition or any feedback process. Based on their TPM (Transcripts Per Million) values, the scatterplots in the figures illustrate the relationship between the expression levels of the genes DDR1 (Discoidin Domain Receptor 1) and two additional biomarkers, CD40 and MAP2K1.

The log2-transformed expression levels of DDR1 are shown on the X-axis, whilst the log2-transformed expression levels of CD40 (Figure 5A) and MAP2K1 (Figure 5B) are shown on the Y-axis. There is a strong negative correlation between DDR1 and CD40 expression levels. As shown by figure 5A, there is correlation coefficient (R) of - 0.74 and highly significant p-value of 1.3e-21. However, figure 5B shows a strong and robust statistically significant positive correlation between DDR1 and MAP2K1, with a R value of 0.77 and a p-value of 0. These findings imply that DDR1 expression is highly correlated with both CD40 and MAP2K1 expression, suggesting possible functional or regulatory connections between these genes that could have consequences for pertinent biological processes.

**Correlation with mesenchymal biomarkers:** The correlation between the expression levels of DDR1 (Discoidin Domain Receptor 1) and different mesenchymal biomarkers is depicted in the plots in figures 6A, B, C, D and

E. Each plot is a scatterplot, with the log2-transformed TPM values of DDR1 on the X-axis and the log2-transformed TPM values of particular mesenchymal biomarkers on the Y-axis. The Pearson correlation coefficient (R) and p-value are given for each plot, indicating the strength and statistical significance of the correlation. High R values (near 1) indicate a strong positive correlation, meaning that as DDR1 expression rises, so does the expression of the corresponding biomarker.

The relationship between a crucial mesenchymal marker, VIM (Vimentin, Y-axis) and DDR1 (X-axis) is displayed in figure 6A. A reasonably strong positive correlation is indicated by the Pearson correlation coefficient (R = 0.69) which implies that increased VIM expression is linked to higher DDR1 expression. The p-value = 0 implies that this correlation is statistically significant. The relationship between DDR1 and TGFβ1 (Transforming Growth Factor Beta 1) is shown in figure 6B.

A strong positive correlation is indicated by the high R value of 0.86. This suggests that DDR1 expression is closely associated with TGFβ1, a critical factor in driving mesenchymal characteristics and processes like EMT. The statistically significant p-value = 0 supports this finding. Similar results were obtained in case of TGFβ3, PXN and ZEB2 and all shows the positive correlation (Figure 6C, D and E).

In conclusion, all plots demonstrate highly significant positive associations between DDR1 and important mesenchymal biomarkers, albeit at different intensities. This implies that DDR1 is crucial for controlling processes associated with mesenchymal tissue, possibly via the MET and EMT pathways.

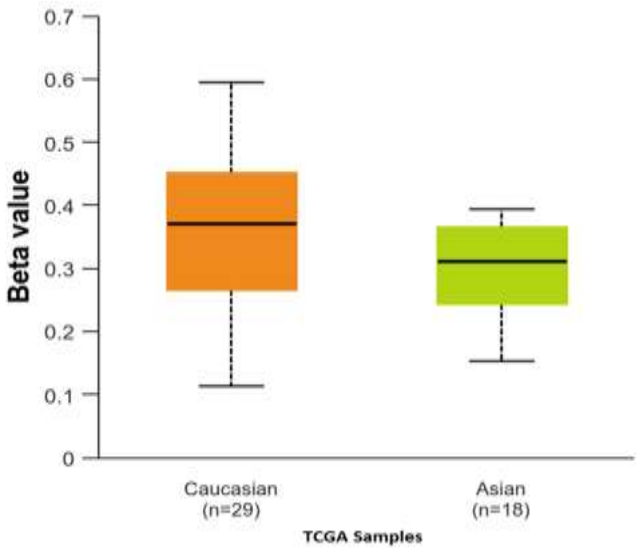


**Figure 4: Promoter Methylation status of DDR1 between Caucasian and Asian cohorts, extracted from DLBC patient population. Beta value represents the degree of methylation.**

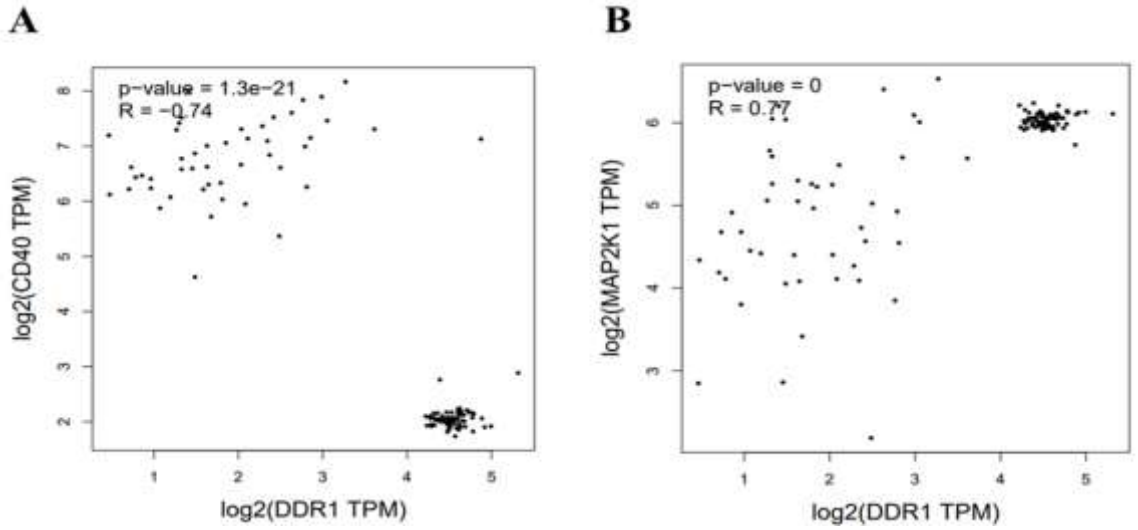


**Figure 5: Correlation Analysis: Spearman Correlation method was used to assess the relationship between DDR1 expression level and other DLBC-known biomarkers (A) Correlation of DDR1 with CD40. (B) Correlation of DDR1 with MAP2K1.**

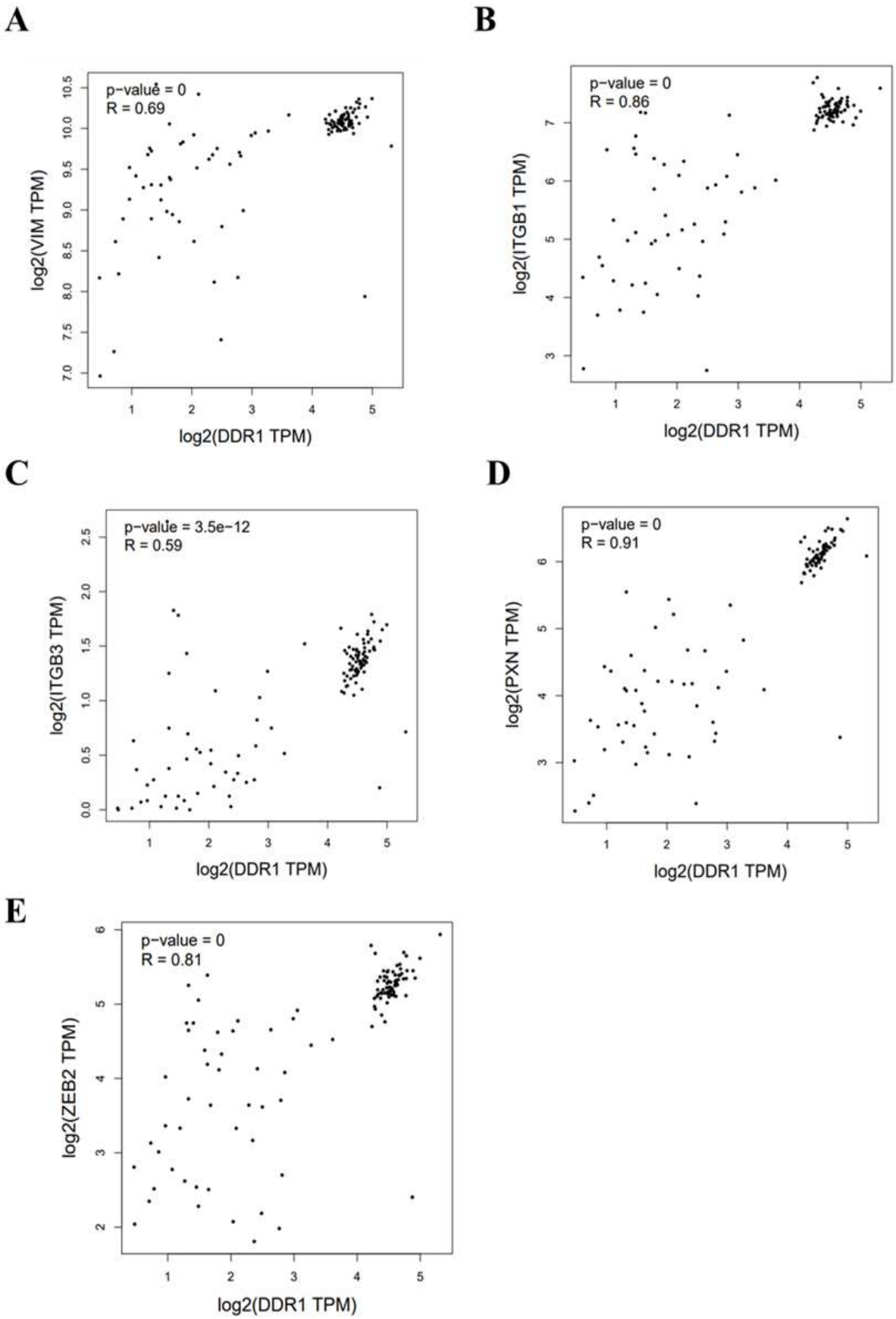


**Figure 6: Correlation Analysis: Spearman Correlation method was used to assess the relationship between DDR1 expression level and Mesenchymal biomarkers (A) Correlation of DDR1 with VIM. (B) Correlation of DDR1 with ITGB1. (C) Correlation of DDR1 with ITGB3. (D) Correlation of DDR1 with PXN. (E) Correlation of DDR1 with ZEB2.**

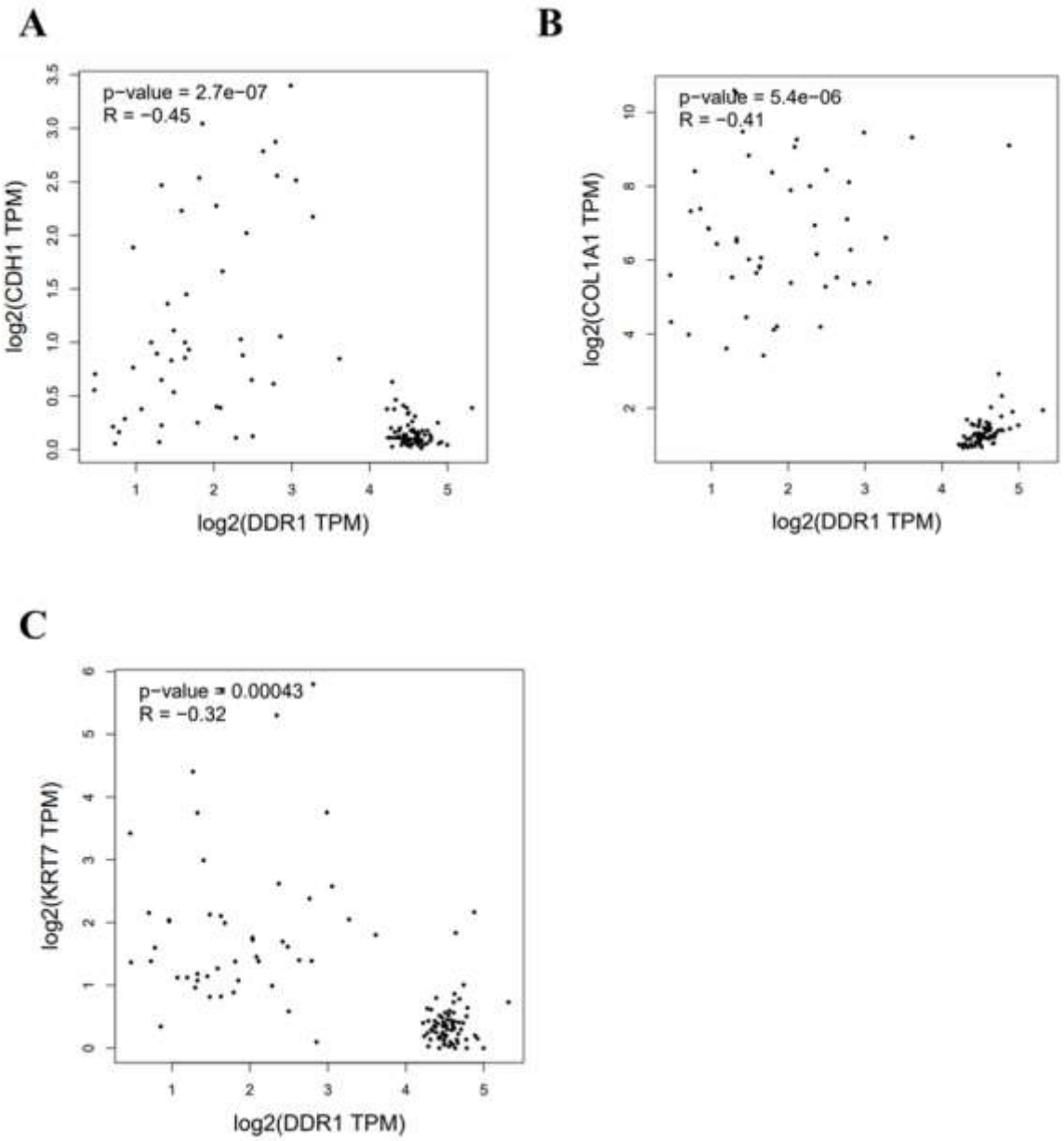


**Figure 7: Correlation Analysis: Spearman Correlation method was used to assess the relationship between DDR1 expression level and Epithelial biomarkers(A) Correlation of DDR1 with CDH1. (B) Correlation of DDR1 with COL1A1. (C) Correlation of DDR1 with KRT7.**

**Correlation with epithelial biomarkers:** The relationship between DDR1 expression and various epithelial markers is determined by the scatterplots in figures 7A, B and C. Figure 7A displays the correlation between DDR1 (X-axis) and CDH1 (E-cadherin, Y-axis). CDH1 is a key epithelial marker that is typically downregulated during epithelial-to-mesenchymal transition (EMT). The p-value (2.7e-07) suggests that this correlation is statistically significant. The Pearson correlation coefficient (R = -0.45) indicates a moderately negative correlation, meaning that higher DDR1 expression is linked to lower CDH1 expression. The association between DDR1 and COL1A1 (Collagen Type I Alpha 1), a mesenchymal marker involved in the extracellular matrix, is examined in figure 7B. The correlation coefficient (R = -0.41) indicates a weak-to-moderate negative correlation, meaning that higher DDR1 expression is associated with lower COL1A1 levels; the p-value (5.4e-06) indicates statistical significance. This is a somewhat surprising result because DDR1 is frequently linked to collagen signalling; additional investigation may be necessary to interpret the negative correlation. The similar results were obtained with KRT7 as well (Figure 7C).

**Interaction Profile of DDR1:** Deciphering the proposed biomarker's signalling pathway is crucial for optimising the site of inhibition for our targeted therapy that uses the biomarker. We can create maps of signalling pathways by linking the hints provided by known interactions. For DDR1 and other recognised mesenchymal and epithelial biomarkers, a molecular interaction map was constructed using STRING. The relationship between DDR1 and other epithelial markers is depicted on the map in figure 8A.

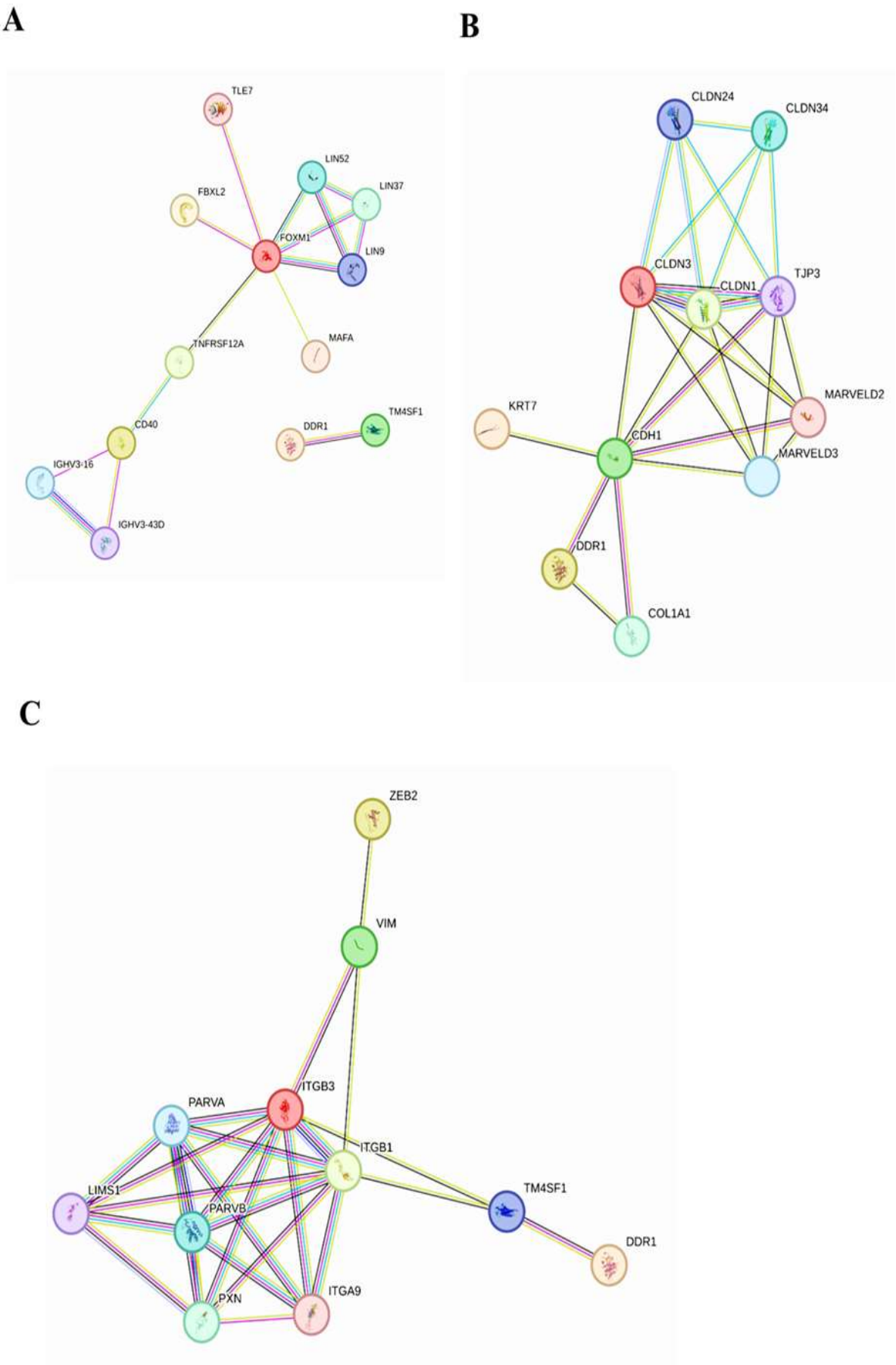


**Figure 8: Co-occurrence of DDR1 with different markers.**
**(A) Co-occurrence of DDR1 with Mesenchymal markers (B) Co-occurrence of DDR1 with Epithelial markers.**

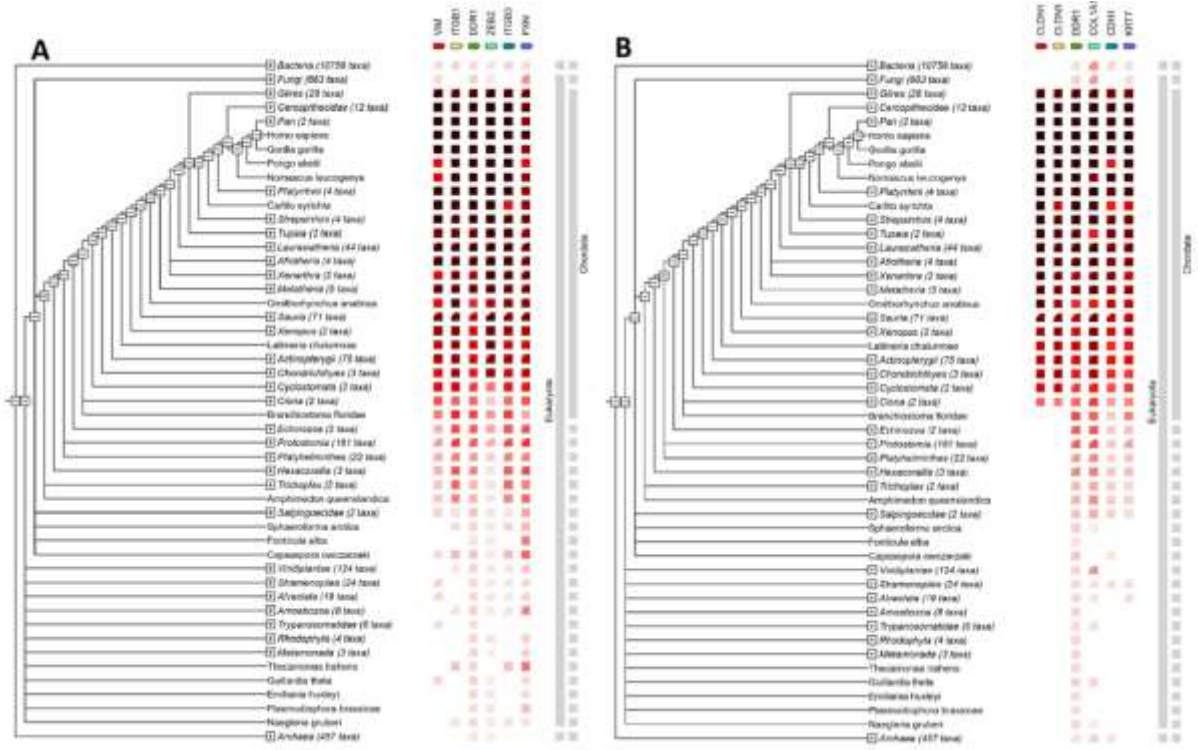


**Figure 9: Interactome Analysis illustrating protein-protein interactions involving DDR1 with highlighted functional clusters representing related biological pathways. (A) Interaction with known biomarkers. (B) Interaction with Epithelial Markers (C) Interaction with Mesenchymal Markers.**

Since DDR1 is overexpressed in DLBC, the map unequivocally demonstrates that it interacts with epithelial biomarkers, which explains how this interaction will promote MET (mesenchymal-epithelial transition). The MET-promoting epithelial biomarkers will aid mesenchymal cells in developing into transition epithelial cells, which will result in secondary tumours[36,48]. CLDN3 and CLDN1 are claudins, which are crucial elements of epithelial cells' tight junctions that preserve cell polarity and barrier function[30]. The relationship that DDR1 has with these indicators implies that it controls tight junction formation or epithelial integrity[30]. Collagen protein COL1A1 is a component of the extracellular matrix. Collagen receptor DDR1 interacts with COL1A1 in a way which is consistent with its function in extracellular matrix signalling and epithelial cell behaviour.

In epithelial cells, KRT7, also known as Keratin 7, is an intermediate filament protein. Its interaction with DDR1 (Figure 8B) suggests that it plays a part in preserving the structure of epithelial cells[35]. A crucial adhesion molecule in epithelial cells, CDH1 also known as E-cadherin preserves both epithelial integrity and cell-cell adhesion. The way that DDR1 interacts with CDH1 suggests that it controls the polarity and adherence of epithelial cells. E-cadherin is frequently downregulated during mesenchymal-epithelial transitions (MET), a process essential for tissue remodelling and the advancement of cancer and DDR1 may influence this process[24]. According to the map in figure 8C, DDR1 (Discoidin Domain Receptor 1) is a node that does not communicate with the other nodes in the network directly. This map shows that DDR1 is isolated, which means that it does not directly interact with the other biomarkers (VIM, ZEB2, ITGB1, ITGB3 and PXN). The VIM (Vimentin), however, is linked to ZEB2, ITGB1 and ITGB3. Additionally, there are strong connections between PXN (Paxillin) and ITGB1 and ITGB3 (Integrin Beta 1 and 3), suggesting that they are involved in the processes of cell adhesion and migration[28].

PXN (Paxillin) interacts with integrins (ITGB1 and ITGB3) and contributes to cytoskeletal organisation, making it a crucial component of focal adhesion dynamics[28]. ZEB2 is linked to VIM and is frequently linked to transcriptional regulation during metastasis and EMT[28]. DDR1 may be a component of an alternative pathway or mechanism that indirectly or specifically influences these mesenchymal biomarkers. In order to analyse the interactions of DDR1, this needs to be investigated or examined further.

**Gene co-occurrence:** The co-occurrence of mesenchymal genes (DDR1, VIM, ITGB1, ITGB3, PXN and ZEB2) across different taxonomic groupings is shown visually in the gene co-occurrence map (Figure 9A), with an emphasis on their prevalence in humans and other species. The co-occurrence of DDR1 with the mesenchymal biomarkers in humans and other taxa is depicted in the heatmap. DDR1 strongly co-occurs (dark red/black boxes) with VIM, ITGB1, ITGB3, PXN and ZEB2 in humans[28]. This implies that in human genomic or transcriptome data, DDR1 and these mesenchymal markers are frequently detected together[7].

Mammals (e.g. Homo sapiens, Pan [chimpanzees] and Gorilla gorilla) have the highest co-occurrence of these genes, suggesting that their functions in higher organisms are conserved. The absence or minimal co-occurrence of these genes in lower taxa, such bacteria or unicellular eukaryotes, emphasises their distinct functions in multicellular processes including migration, cell adhesion and epithelial-to-mesenchymal transition (EMT).

With an emphasis on Homo sapiens, the gene co-occurrence map of epithelial biomarkers (COL1A1, CLDN1, CDH1, KRT7 and CLDN3) across different taxonomic groupings is displayed (Figure 9B). In mammals and closely similar species, DDR1 is frequently found co-occurring with these biomarkers, suggesting that these interactions are conserved in higher organisms. The biomarkers are either non-existent or weakly co-occurring in more distant taxa such as bacteria or lower eukaryotes, indicating that these genes have evolved particular roles in the biology of the epithelium in complex creatures[3].

The significant gene cooccurrence with both mesenchymal and epithelial biomarkers in humans, may, therefore, play a role in transition between mesenchymal and epithelial state of cells[47]. It is very important to understand that there is a transition state or quasi state between these two phenotypes and this state is marked by specific markers in equilibrium, because of this state we found DDR1 to be in co-occurrence with both the phenotypes. However, it is quite evident from the stage plot that quasi state further takes epithelial form to seed and colonize and leads to formation of secondary tumors[47].

## Conclusion

Datasets and Kaplan-Meier survival analyses show that DDR1 is overexpressed in stage 4 DLBC. This upregulation correlates with aggressive tumour characteristics, indicating a role in promoting invasion and metastasis, even though it has no discernible effect on overall or disease-free survival. Additionally, DDR1 controls immune cell infiltration and has therapeutic promise, especially for boosting the effectiveness of immunotherapy. Elevated DDR1 expression was found in all carcinoma samples by the pan-cancer study, with stage 4 DLBC showing a significant increase. DDR1 overexpression promotes the mesenchymal-epithelial transition (MET), which enables mesenchymal cells to colonise secondary sites and aids in the formation of metastatic tumours.

According to promoter methylation studies, the Asian population had hypomethylation, which was linked to higher DDR1 expression and MET activation. This emphasises how epigenetic variables control DDR1 expression and tumour development. There were substantial negative connections with DDR1 and high positive correlations with mesenchymal markers (including VIM, TGFβ1, PXN and ZEB2) and epithelial indicators (like CDH1). According to these correlations, DDR1 plays a dual function in controlling mesenchymal and epithelial transitions which influences adhesion, cell migration and tumour metastasis. DDR1's role in MET was highlighted by STRING analysis, which also revealed its interaction with epithelial markers such as CLDN1 and CDH1.

Even though DDR1 seems to be isolated in its network of mesenchymal biomarkers, it probably affects these indicators in different ways, thus more research is necessary. DDR1's evolutionary conservation in intricate processes like MET and EMT is supported by its continuous co-occurrence with mesenchymal and epithelial indicators in animals. The dual regulatory role of DDR1 in tumour growth and metastasis is highlighted by this co-occurrence.

In conclusion, DDR1 is an important biomarker in DLBC that affects immunological regulation, tumour growth and treatment response. It provides important insights into MET and EMT processes through its interactions with mesenchymal and epithelial pathways. To fully understand its specific molecular mechanisms and therapeutic possibilities in the management of DLBC, extensive research is required.

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## References

1. Abdulhussein R., McFadden C., Fuentes-Prior P. and Vogel W.F., Exploring the collagen-binding site of the DDR1 tyrosine kinase receptor, *Journal of Biological Chemistry*, **279(30)**, 31462-31470 **(2004)**

2. Alizadeh A.A., Eisen M.B., Davis R.E., Ma C., Lossos I.S., Rosenwald A. and Staudt L.M., Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling, *Nature*, **403(6769)**, 503-511 **(2000)**

3. Baltes F., Caspers J., Henze S., Schlesinger M. and Bendas G., Targeting discoidin domain receptor 1 (DDR1) signaling and its crosstalk with β1-integrin emerges as a key factor for breast cancer chemosensitization upon collagen type 1 binding, *International Journal of Molecular Sciences*, **21(14)**, 4956 **(2020)**

4. Bibi S., Zeng W., Zheng P., Mehmandousti S.M.M. and Jin T., Discoidin Domain Receptor 1 as a potent therapeutic target in solid tumors, *hLife*, **2(9)**, 454-466 **(2024)**

5. Chandrashekar D.S., Bashel B., Balasubramanya S.A.H., Creighton C.J., Ponce-Rodriguez I., Chakravarthi B.V. and Varambally S., UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses, *Neoplasia*, **19(8)**, 649-658 **(2017)**

6. Chandrashekar D.S., Karthikeyan S.K., Korla P.K., Patel H., Shovon A.R., Athar M. and Varambally S., UALCAN: An update

to the integrated cancer data analysis platform, *Neoplasia*, **25**, 18-27 **(2022)**

7. Chen Y.L. et al, Discoidin domain receptor-1 (DDR1) is involved in angiolymphatic invasion in oral cancer, *Cancers*, **12(4)**, 841 **(2020)**

8. Coiffier B., Lepage E., Brière J., Herbrecht R., Tilly H., Bouabdallah R. and Gisselbrecht C., CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma, *New England Journal of Medicine*, **346(4)**, 235-242 **(2002)**

9. Datta A., Deng S., Gopal V., Yap K.C.H., Halim C.E., Lye M.L. and Yap C.T., Cytoskeletal dynamics in epithelial-mesenchymal transition: insights into therapeutic targets for cancer metastasis, *Cancers*, **13(8)**, 1882 **(2021)**

10. Dawoud M.M., Salah M. and Mohamed A.S.E.D., Clinical significance of immunohistochemical expression of DDR1 and β-catenin in colorectal carcinoma, *World Journal of Surgical Oncology*, **21(1)**, 168 **(2023)**

11. Erdem D., Büyüksimsek M., Gunaldim M. and Sahin N., DDR1 may be a good biomarker for brain tumors, *Journal of Clinical Oncology*, **37(15)**, e13557 **(2019)**

12. Erdem D., Gunaldi M., Karaman I., Adilay U., Yılmaz İ.U., Eseoglu M. and Gunaldi O., Discoidin domain receptor 1 as a promising biomarker for high-grade gliomas, *Journal of Cancer Research and Therapeutics*, **19(Suppl 1)**, S285-S293 **(2023)**

13. Ford C.E., Lau S.K., Zhu C.Q., Andersson T., Tsao M.S. and Vogel W.F., Expression and mutation analysis of the discoidin domain receptors 1 and 2 in non-small cell lung carcinoma, *British Journal of Cancer*, **96(5)**, 808-814 **(2007)**

14. Fu H.L., Valiathan R.R., Arkwright R., Sohail A., Mihai C., Kumarasiri M. and Fridman R., Discoidin domain receptors: unique receptor tyrosine kinases in collagen-mediated signaling, *Journal of Biological Chemistry*, **288(11)**, 7430-7437 **(2013)**

15. Gadiya M. and Chakraborty G., Signaling by discoidin domain receptor 1 in cancer metastasis, *Cell Adhesion & Migration*, **12(4)**, 315-323 **(2018)**

16. Gao Y., Zhou J. and Li J., Discoidin domain receptors orchestrate cancer progression: A focus on cancer therapies, *Cancer Science*, **112(3)**, 962-969 **(2021)**

17. Henriet E., Sala M., Abou Hammoud A., Tuariihionoa A., Di Martino J., Ros M. and Saltel F., Multitasking discoidin domain receptors are involved in several and specific hallmarks of cancer, *Cell Adhesion & Migration*, **12(4)**, 363-377 **(2018)**

18. Iqbal J., Wright G., Wang C., Rosenwald A., Gascoyne R.D., Weisenburger D.D. and Staudt L.M., Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphoma, *Blood, The Journal of the American Society of Hematology*, **123(19)**, 2915-2923 **(2014)**

19. Kalluri R. and Weinberg R.A., The basics of epithelial-mesenchymal transition, *The Journal of Clinical Investigation*, **119(6)**, 1420-1428 **(2009)**

20. Kim H.G., Tan L., Weisberg E.L., Liu F., Canning P., Choi H.G. and Gray N.S., Discovery of a potent and selective DDR1 receptor tyrosine kinase inhibitor, *ACS Chemical Biology*, **8(10)**, 2145-2150 **(2013)**

21. Li S., Young K.H. and Medeiros L.J., Diffuse large B-cell lymphoma, *Pathology*, **50(1)**, 74-87 **(2018)**

22. Li T., Hu H., Song Y., Shi Y., Hu D., Shen W. and Ning B., DDR1 is a Novel Biomarker and Potential Therapeutic Target for the Combination Treatment of Liver Hepatocellular Carcinoma, *Cancer Control*, **31**, 10732748241286257 **(2024)**

23. Liu J., Chiang H.C., Xiong W., Laurent V., Griffiths S.C., Dülfer J. and Zhang N., A highly selective humanized DDR1 mAb reverses immune exclusion by disrupting collagen fiber alignment in breast cancer, *Journal for Immunotherapy of Cancer*, **11(6)**, e006720 **(2023)**

24. Liu X. and Chu K.M., E-cadherin and gastric cancer: cause, consequence and applications, *BioMed Research International*, **2014(1)**, 637308 **(2014)**

25. Lossos I.S. and Morgensztern D., Prognostic biomarkers in diffuse large B-cell lymphoma, *Journal of Clinical Oncology*, **24(6)**, 995-1007 **(2006)**

26. Margielewska S.K., The contribution of discoidin domain receptor 1 to the pathogenesis of diffuse large B cell lymphoma, Doctoral dissertation, University of Birmingham **(2018)**

27. Martelli M. et al, Diffuse large B-cell lymphoma, *Critical Reviews in Oncology/Hematology*, **87(2)**, 146-171 **(2013)**

28. Ostrowska-Podhorodecka Z., Ding I., Norouzi M. and McCulloch C.A., Impact of vimentin on regulation of cell signaling and matrix remodeling, *Frontiers in Cell and Developmental Biology*, **10**, 869069 **(2022)**

29. Pal R., Kumaraswamy B., Md. Ashadul S.K., Hosamani K.R. and Swamy Purawarga Matada G., Advancement of DDR1 and DDR2 Inhibitors: Therapeutic Potential of Bioactive Compounds, Designing Strategies and Structure-Activity Relationship (SAR), *Chemistry Select*, **9(30)**, e202401216 **(2024)**

30. Raya-Sandino A., Lozada-Soto K.M., Rajagopal N., Garcia-Hernandez V., Luissint A.C., Brazil J.C. and Nusrat A., Claudin-23 reshapes epithelial tight junction architecture to regulate barrier function, *Nature Communications*, **14(1)**, 6214 **(2023)**

31. Riggio A.I., Varley K.E. and Welm A.L., The lingering mysteries of metastatic recurrence in breast cancer, *British Journal of Cancer*, **124(1)**, 13-26 **(2021)**

32. Sarma S. and Mehta J., Spectrum of lymphomas in India, *International Journal of Molecular and Immuno Oncology*, **9(1)**, 16-24 **(2024)**

33. Sehn L.H. and Salles G., Diffuse large B-cell lymphoma, *New England Journal of Medicine*, **384(9)**, 842-858 **(2021)**

34. Shrivastava A., Radziejewski C., Campbell E., Kovac L., McGlynn M., Ryan T.E. and Yancopoulos G.D., An orphan

receptor tyrosine kinase family whose members serve as nonintegrin collagen receptors, *Molecular Cell*, **1(1)**, 25-34 **(1997)**

35. Stenvall C.G.A., Nyström J.H., Butler-Hallissey C., Jansson T., Heikkilä T.R., Adam S.A. and Toivola D.M., Cytoplasmic keratins couple with and maintain nuclear envelope integrity in colonic epithelial cells, *Molecular Biology of the Cell*, **33(13)**, ar121 **(2022)**

36. Szklarczyk D., Gable A.L., Lyon D., Junge A., Wyder S., Huerta-Cepas J. and Mering C.V., STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets, *Nucleic Acids Research*, **47(D1)**, D607-D613 **(2019)**

37. Tang Z., Kang B., Li C., Chen T. and Zhang Z., GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis, *Nucleic Acids Research*, **47(W1)**, W556-W560 **(2019)**

38. Tang Z., Li C., Kang B., Gao G., Li C. and Zhang Z., GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, *Nucleic Acids Research*, **45(W1)**, W98-W102 **(2017)**

39. Thiery J.P. and Sleeman J.P., Complex networks orchestrate epithelial–mesenchymal transitions, *Nature Reviews Molecular Cell Biology*, **7(2)**, 131-142 **(2006)**

40. Tomczak K., Czerwińska P. and Wiznerowicz M., Review The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge, *Contemporary Oncology/Współczesna Onkologia*, **2015(1)**, 68-77 **(2015)**

41. Wang J., Walker R.L., Hornicek F.J., Shi H. and Duan Z., Inhibition of discoidin domain receptor 1 (DDR1) as a new therapeutic strategy for osteosarcoma, *The FASEB Journal*, **38(23)**, e70239 **(2024)**

42. Wang S. et al, Discoidin domain receptor 1 is a potential target correlated with tumor invasion and immune infiltration in gastric cancer, *Frontiers in Immunology*, **13**, 933165 **(2022)**

43. Weiner H.L., Huang H., Zagzag D., Boyce H., Lichtenbaum R. and Ziff E.B., Consistent and selective expression of the discoidin domain receptor-1 tyrosine kinase in human brain tumors, *Neurosurgery*, **47(6)**, 1400-1409 **(2000)**

44. Xiao Q., Jiang Y., Liu Q., Yue J., Liu C., Zhao X. and Ge G., Minor type IV collagen α5 chain promotes cancer progression through discoidin domain receptor-1, *PLoS Genetics*, **11(5)**, e1005249 **(2015)**

45. Yang J. and Weinberg R.A., Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis, *Developmental Cell*, **14(6)**, 818-829 **(2008)**

46. Yang L., Zhang Y., Tang Y., Wang Y., Jiang P., Liu F. and Feng N., A pan-cancer analysis of DDR1 in prognostic signature and tumor immunity, drug resistance, *Scientific Reports*, **13(1)**, 5779 **(2023)**

47. Yao D., Dai C. and Peng S., Mechanism of the mesenchymal–epithelial transition and its relationship with metastatic tumor formation, *Molecular Cancer Research*, **9(12)**, 1608-1620 **(2011)**

48. Zeisberg M. and Neilson E.G., Biomarkers for epithelial-mesenchymal transitions, *The Journal of Clinical Investigation*, **119(6)**, 1429-1437 **(2009)**.

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