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DESMOCOLLIN 3 GENE (DSC3) EXPRESSION AND CANCER - *IN SILICO* EVALUATION

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ABSTRACT

Desmocollin-3 (DSC3) is DSC3 protein coding gene, expressed in some epithelial cancers. DSC3 expression has prognostic value in some cancers. Using cBioportal and Kaplan–Meier plotter DSC3 gene was evaluated in 12 cancers using TCGA data and compared with Hallmark gene expression. Cancers were grouped to have low (Group I), intermediate (Group II) and high (Group III) DSC3 expression. Higher DSC3 expression in group III is associated with better survival and in group I with poor survival. DSC3 expression correlated with desmosomal genes DSG3, DSC1, DSG1, PKP1 in spite of its known reciprocal relationship with DSC1, DSG1 and PKP1 under physiological conditions. Of 10 Hallmarks evaluated, three hallmarks had no correlation with DSC3. Activating invasion and metastasis (AIM) (genes SERPINB5, PERP, SNAI2) and Evading Growth Suppressors (EGS) (gene TP63) were the key hallmarks associated with DSC3 expression. Gene expression correlation with DSC3 across various hallmark genes reveals no correlation in group I cancers. Our finding demonstrate that higher DSC3 expression level may be useful for prognostication and needs further evaluation. The finding of this analysis also suggests critical role of TP63 in DSC3 expressing cancers and potentially a different biological process unique to DSC3 expressing cancers.

Keywords: Cancer, DSC3 expression, Prognosis, Hallmark of cancer

Introduction

Cancer arising from epithelial tissues (carcinoma) encompass 80-90% of all cancers (SEER Training Modules, 2020). In epithelia, the cells are adherent to each other via cadherin proteins located on lateral wall of epithelial cells (adherence junctions and desmosomes) to ensure tissue integrity and mechanical strength to withstand stress and strain (Gumbiner 1986). Cadherin proteins play an important role in bidirectional communication (through signalling pathways) between adjacent cells and surroundings (Klezovitch & Vasioukhin 2015). They also regulate proliferation, polarity, migration and differentiation of epithelial cells and are responsible for formation, morphogenesis, maturation and homeostasis of epithelia. Epithelial cancers are known for abnormal expression of cadherin protein. E cadherin is widely studied and its loss is associated with epithelial mesenchymal transformation.

Desmocollin 3 (DSC3) is a calcium dependent desmosomal cadherin. It is the only cadherin which is p53 responsive (Cui *et al*., 2011) as well as tumour suppressive like(Cui *et al.,* 2012). It is used as a diagnostic biomarker to differentiate squamous lung cancer and adenocarcinoma of lung (Tsuta *et al.,* 2011). Expression of DSC3 protein is known in squamous non-small cell lung carcinoma (NSCLC), melanoma, ovarian cancer, bladder cancer, pancreatic cancer, esophageal cancer, cervical cancer, chondrosarcoma, oral cancer and colorectal cancer (Valentina *et al.,* 2009, Khamar 2017, Salerno *et al.,* 2016, Uhlen *et al.,* 2010, Uhlen *et al.,* 2017, Robertson *et al.,*2017, Wang *et al.,*2014, Fitzgerald *et al.,*2011, Wang *et al.,* 2011, Knösel *et al.,*2013). Its prognostic value is perplexing with better prognosis in squamous NSCLC (Belani *et al.,*2017, Cui *et al.,*2012) and poor prognosis in ovarian cancer.(Salerno *et al.,*2016).

Hallmark of cancers proposed by Hanahan and Weinberg organizes cancers with phenotypical and genotypical diversity into logical set of underlying organizing principles using knowledge about cell biology, histopathology, biochemistry, immunology, and pharmacology (Hanahan & Weinberg 2000). Each hall mark is assigned with set of genes (Hanahan & Weinberg 2011, Iannuccelli *et al.,*2020). Information about genetic alteration in various cancers is available in The Cancer Genome Atlas (TCGA) database (Weinstein *et al.,*2013). Various tools have been developed to analyse The TCGA. cBioportal (Gao *et al.,*2013) and Kaplan Meier (K – M) plotter (Nagy *et al.,*2013) are two such tools. cBioportal is useful for assessment of gene expression, genetic alteration and gene correlation. The K-M plotter dataset is helpful for studying relationship between gene expression and survival in 12 cancers.

We undertook this study to evaluate for DSC3 gene expression in various cancer and, its relationship with survival, other desmosomal genes and hallmark genes using, cBioportal and K - M plotter tools.

Methods

Study characteristics: We have used cBioportal (cbioportal.org), and K – M

Plotter (kmplotter.org) data for studying DSC3 expression and its relationship with various parameters. A total of 12 cancer studies (Table S1), for which data is available in both datasets, were studied using as follows:

cBioportal

DSC3 gene expression view of TCGA dataset:

The level of DSC3 expression in tumour samples and change from normal tissue is analysed by using the Gene Expression Viewer tool [http://firebrowse.org/viewGene.html?gene=DSC3] (Fire Browse, Broad Institute of MIT and Harvard, 2019) which provides RNA-Seq by Expectation Maximization (RSEM) data for DSC3 in various cancer. The dataset filtered by selecting the type of cancer to view the RSEM data for DSC3 in cancer and in normal tissue (Gao *et al.,* 2013). The esophageal carcinoma (ESCA) study included both adenocarcinoma and squamous cell carcinoma population, the RSEM data of individual population was extracted from cBioportal data set.

DSC3 Gene alteration analysis:

DSC3 RNA expressions are extracted from databases available at cBioPortal.org for Cancer Genomics. The query by gene "DSC3" was submitted in TCGA pancancer atlas datasets available (Table S1, 4974 human samples, from different cancer studies of TCGA Research Network (https://www.cbioportal.org/) (Gao *et al.,* 2013). The 'cancer type summary' ribbon showed the alteration frequency of the gene in various cancers.

Co-expression analysis:

The 'Co-expression' ribbon for each cancer correlate the DSC3 mRNA expression with mRNA expression of other proteins. The spearman's correlation is determined for each sample by plotting DSC3 mRNA expression on X-axis and co-expressing gene on Y-axis. We have extracted the spearman's correlation for DSC3 with other desmosomal molecules and hallmarks of cancer (Hanahan & Weinberg 2011, Iannuccelli *et al.,*2020) for each cancer study. For gene correlation a spearman correlation value of ≥ 0.5 was considered meaningful, and > 0.7 considered as highly correlated. (Table S4B**)**. The TCGA data set of ESCA study include both adenocarcinoma (ESACA) and squamous cell carcinoma (ESSCA) population. RSEM data of individual population extracted for all the genes. The spearman correlation value with DSC3 was calculated by RSEM data each gene using online statistical (https://www.socscistatistics.com/tests/spearman/default2.aspx) (Jeremy Stangroom, Social Science Statistics, Updated 2018).

On basis of correlation values, the heat map and clustering of groups are assessed by (http://heatmapper.ca/expression/) (Babicki *et al.,* 2016), a web tool for visualizing clustering of multivariate data. The spearman's correlation values are uploaded to the tool and the Euclidean distance is measured on basis of average linkage in clustering method. The scale

type is kept as none. The clustering is applied to both rows and column.

K-M Plotter Survival analysis and Hazard Ratios estimations:

The mRNA seq dataset of 'DSC3' gene symbol is analysed for survival assessment using auto select best cut-off option where all possible cut off values between the lower and upper quartiles are computed. Using auto select best cutoff option for DSC3, median overall survival (OS) was evaluated for all cancers. The Kaplan-Meier plot is drawn after choosing the cancer types (Nagy *et al.,* 2018). The Log rank test is used as statistical inference between the two groups. P value of < 0.05 was considered statistically significant.

Results

Study characteristics.

DSC3 expression was evaluated using online platforms (cBioPortal and KM-Plotter) in twelve (12) cancer types available on both. (Table 1 and S1)

DSC3 expression in various cancer

DSC3 expression level in twelve evaluated cancers vary in their median mRNA expression of from 1.5 in Kidney papillary cell carcinoma (KIPAN) to 19962 in ESSCA. Based on expression levels, cancers were segregated into 3 groups. cancer with low expression – KIPAN, Liver hepatocellular carcinoma (LIHC), Lung Adenocarcinoma (LUAD), Pancreatic adenocarcinoma (PAAD), ESACA, Ovarian serous cystadenocarcinoma (OV) (RSEM value:1 – 100; group I); intermediate expression - Breast invasive carcinoma (BRCA), Bladder urothelial carcinoma (BLCA) (RSEM value:100 – 1000; group II); and high expression - Lung Squamous cell carcinoma (LUSC) Cervical squamous cell carcinoma (CESC) Head and Neck Squamous cancer (HNSC) and ESSCA (RSEM value: >1000; group III). (Table 1).

DSC3 expression in comparison to Normal

KIPAN, LIHC, LUAD, ESACA, BRCA, and HNSC were found to have reduced expression of DSC3 (Normal/DSC3 < 1) compared to normal while ESSCA, LUSC and CESC had >10-fold increase in expression of DSC3 compared to normal. BLCA and PAAD also had increase in DSC3 expression by 1.6 fold and 3.8 fold respectively. This could not be analysed for OV as expression level in normal tissue was not available. (Table 1).

Mutation levels of DSC3 TP53 and TP63:

Number of patients harbouring DSC3 mutation ranged from 0.2% – 4.2% of samples in various cancers. The mutational rate was highest for TP53 followed by T63 and DSC3 respectively (Table S2).

DSC3 expression as prognostic marker for survival in various cancer (K-M plotter)

K-M plotter identified cutoff vlue of DSC3 expression for each cancer separately based on survival and divided them in two cohort with DSC3 expresion higher or lower than cut off value. Comparison of cohorts with values higher or lower than cut off provided hazard ratio for each cancer separately. The cohort with DSC3 expression more than cut-off value had a poor survival in Group I and better

survival in Group III. Cut-off value was also found to be highest in group III and lowest in group I. There was no correlation between mean, median and cut off values for DSC3 expression (Table 2).

Correlation of DSC3 with other desmosomal components

Amongst desmosomal components, significant correlation (>0.5) with DSC3 was seen only in group II and group III (Table S3). Highest correlation of DSC3 was observed with DSG3 followed by DSC2, DSG1 and DSC1 respectively. Amongst armadillo proteins PKP1 was highly correlated followed by JUP and PKP3. Cluster analysis of these genes displayed difference in pattern of clustering across groups (Fig. 1). In group III, DSG2 and PKP2 were in one cluster while in group II, they were accompanied by DSG4, JUP, and PKP3. In group I, both DSG2, PKP2 were far distanced in different clusters. Similarly, DSG1 and PKP1 are clustered together in group II and III but not in group I. (Fig. 1).

DSC3 co-relation with hallmarks of cancer

Across 10 Hallmarks of cancer, 215 assigned genes to them were evaluated for correlation with DSC3 (Table 4A). Of 215 genes evaluated, 20 genes assigned to seven hallmarks were correlated (spearman correlation value ≥ 0.5) with DSC3 (Table S4B). None of the genes in 3 hallmarks - Genome instability and mutation (GIM), Deregulating cellular energies (DCE) and Avoiding immune destruction (AID) correlated with DSC3 (Table S4B). Of remaining 7 hallmarks, significant correlation (≥ 25% of cancers) with DSC3 was seen in Activating invasion and metastasis (AIM; genes SERPINB5, PERP and SNAI2) and Evading growth Suppressors (EGS; gene TP63). No correlation between any hallmark and group I cancer was seen. Of the three genes belonging to AIM hallmark, SERPINB was correlated in all cancers of group II and III (Table 4SB), PERP correlated in group III and CDH3 in group II. A high correlation (Spearman value >0.7) of TP63 and PERP was observed in CESC, and SERPINB in BRCA.

Heatmap analysis of DSC3 correlated genes displayed difference in hierarchical clustering among the groups (Fig. 2). In group I, TP63 was clustered with SNAI2, followed by CDH3 (AIM) and SERPINB5. PERP belong to other cluster. In group II, PERP and TP63 were in same cluster closer to SNAI2, and CDH3 respectively. SERPINB5 also fall in same cluster but distanced from these gene groups. In group III, all the 3 genes (TP63, PERP, SERPINB5) were found nearest to each other as single group in positive cluster. SNAI2 belong to other cluster.

Negatively correlated genes formed a separate cluster in group II and III. Group I displayed mosaic clustering of these genes (Fig. 2). CDH1 (AIM) found negative in group II, in place of PDIA6 (AIM) in group III, rest other genes were common in group II and III.

Table 1: DSC3 mRNA levels in various tumour tissue

DSC3 RNA Seq V2												
		Tumor Tissue				Normal Tissue				Fold Change		
Group	Cancer	Cases	Median	1st Q	3rd Q	Cases	Median	1st Q	3rd Q	Median	1st Q	3rd Q
Group 1- 0.1 – 100 (Low Expression; L)	KIPAN	720	1.5	0.7	3.6	121	2.3	1.2	4.2	0.64	0.6	0.9
	LIHC	231	2.0	0.9	6.1	42	3.9	2.0	8.5	0.52	0.5	0.7
	LUAD	496	10	3.6	38	54	18.1	5.1	37.5	0.56	0.7	1.0
	PAAD	176	46	17	111	4	12.0	8.9	16.1	3.80	1.9	6.9
	*ESACA	87	68	14	493	10	202	44	976	0.07	0.3	0.5
	OV	307	86	29	223	NA	NA	NA	NA	-	-	-
Group 2- 100 – 1000 (Intermediate Expression; I)	BRCA	1090	120	22	455	111	1097	690	1663	0.11	0.0	0.3
	BLCA	394	639	29	3566	18	402	260	1176	1.59	0.1	3.0
Group 3- >1000 (High Expression; H)	LUSC	501	6208	2896	10086	51	33	16	76	195	184.8	133.4
	CESC	306	10086	495	18820	3	48	23	75	209	21.7	250.7
	HNSC	522	15287	10086	23170	44	16384	7643	21619	0.93	1.3	1.1
	*ESSCA	95	19962	12505	28423	10	202	44	976	98.8	284.2	29.1

* Normal sample expression from esophageal carcinoma (ESCA) was used for both ESACA and ESSCA.

Table 2: DSC3 expression as prognostic marker for survival in various cancer (K-M plotter)

Group	Cancer (N)	Cut-off	% of patients		Median Survival (months)		HR (95% CI)	Log Rank (p value)
			> Cut off	< Cut off	> Cut off	< Cut off		
Group 1 (L)	KIPAN (287)	5	27.9	72.1	45.7	77.7	1.63 (0.86 - 3.1)	0.13
	LIHC (370)	0.1	73.8	26.2	46.2	84.4	1.66 (1.09 - 2.51)	0.016
	LUAD (504)	13	51.4	48.4	41.9	54.4	1.34 (1.0 - 1.8)	0.05
	PAAD (177)	31	65.5	34.5	18.7	37.7	2.06 (1.27 - 3.32)	0.0027
	*ESACA (80)	148	50	50	18.6	32	2.02 (1.04 – 3.94)	0.035
	OV (373)	228	26.3	73.7	41.6	46.0	1.22 (0.92 - 1.61)	0.17
Group 2 (I)	BRCA (1089)	32	74.8	25.2	142.2	113.6	0.61 (0.43 - 0.86)	0.0047
	BLCA (404)	3235	27.2	72.8	17.0	47.4	1.97 (1.47 - 2.68)	8.6E-06
Group 3 (H)	*ESSCA (81)	17458	67.9	32.1	42.1	22.7	0.6 (0.27 - 1.35)	0.22
	LUSC (495)	6274	59.2	40.8	72.0	36.9	0.64 (0.49 - 0.84)	0.0011
	CESC (304)	6152	62.8	37.2	41.5	23.8	0.64 (0.4 - 1.03)	0.063
	HNSC (499)	24083	25.3	74.7	70.7	48.6	0.71 (0.52 - 0.98)	0.037

* Expression range is extracted from K-M plotter data set and grouped on basis of cut-off value.

Fig. 1: Cluster analysis of spearman correlation of DSC3 with Desmosomal molecules.

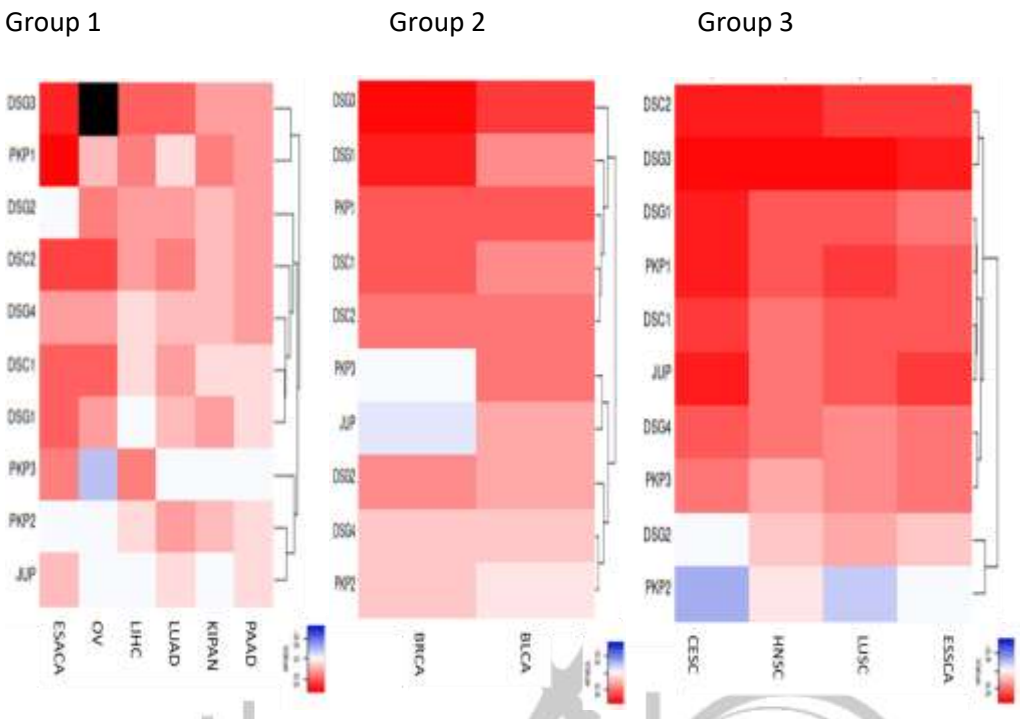
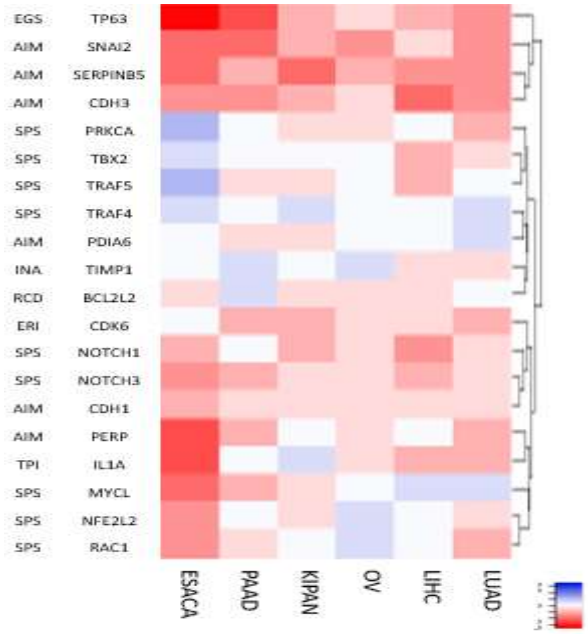
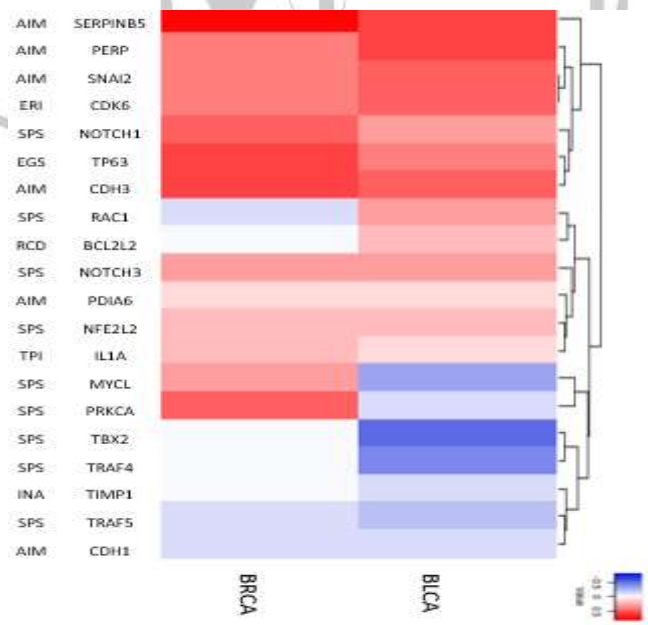


Fig. 2: Cluster analysis of spearman correlation (≥ 0.5) of DSC3 with hallmarks of cancer molecules.

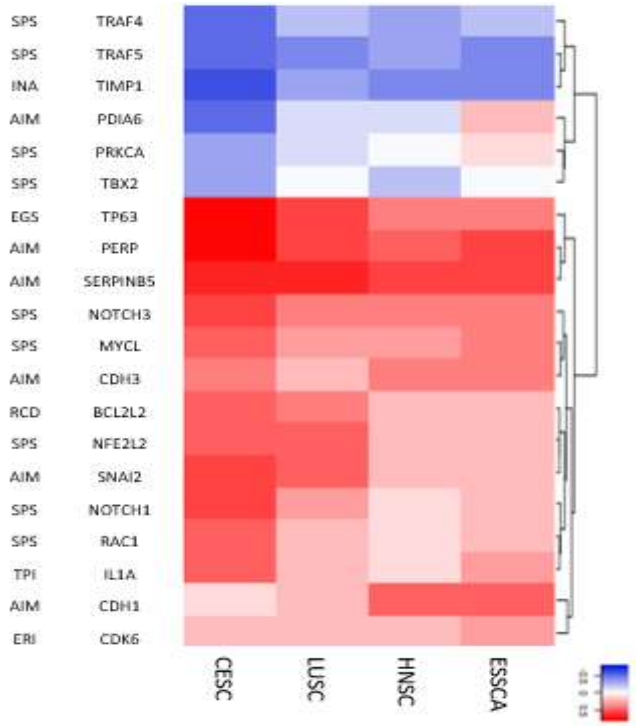
Group 1



Group 2



Group 3


SPS TRAF4
SPS TRAF5
INA TIMP1
AIM PDIA6
SPS PRKCA
SPS TBX2
EGS TP63
AIM PERP
AIM SERPINB5
SPS NOTCH3
SPS MYCL
AIM CDH3
RCD BCL2L2
SPS NFE2L2
AIM SNAI2
SPS NOTCH1
SPS RAC1
TPI IL1A
AIM CDH1
ERI CDK6
CESC
LUSC
HNSC
ESSCA

Supplementary Appendix:

This appendix has been provided by the authors to give readers additional information about their work.

Table of Contents

Table S1: Cancer studies analysed in this article

TCGA PanCancer Atlas	Initial	Sample No. –Gene expression viewer	Sample No. - cBioPortal	Sample No. -K-M Plotter
Kidney renal papillary cell carcinoma	KIPAN	720	283	287
Liver hepatocellular carcinoma	LIHC	231	372	370
Lung adenocarcinoma	LUAD	496	566	504
Pancreatic adenocarcinoma	PAAD	176	184	177
Esophageal adenocarcinoma	ESACA	-	87	80
Ovarian serous cystadenocarcinoma	OV	307	585	373
Breast invasive carcinoma	BRCA	1090	1084	1089
Bladder urothelial carcinoma	BLCA	394	411	404
Lung Squamous cell carcinoma	LUSC	501	487	495
Cervical squamous cell carcinoma	CESC	306	297	304
Head & Neck Squamous cancer	HNSC	522	523	499
Esophageal Squamous carcinoma	ESSCA	-	95	81

Table S2: DSC3 structural alteration in various cancer

Cancer	Sample No.	Mutation			Deep Deletion			Amplification			Multiple Alterations		
		DSC3	TP63	TP53	DSC3	TP63	TP53	DSC3	TP63	TP53	DSC3	TP63	TP53
KIPAN	283	1.1	0.4	3.2	0.4	NR	0.7	NR	0.7	NR	NR	NR	NR
LIHC	372	0.3	0.8	28.5	NR	NR	1.3	0.3	0.8	0.3	NR	0.3	1.1
LUAD	566	4.2	2.3	51.8	0.2	0.7	0.2	0.5	1.6	0.2	NR	0.2	1.4
PAAD	184	0.5	0.5	54.4	1.6	NR	0.5	3.8	1.6	1.1	NR	NR	4.4
ESACA	87	1.2	NR	79.3	2.3	NR	NR	2.3	5.8	NR	NR	NR	1.2
OV	585	0.3	0.5	60.6	0.5	0.2	0.3	3.6	15.4	1.9	NR	0.2	3.3
BRCA	1084	0.7	0.4	31.6	NR	0.1	0.7	0.7	1.6	0.2	NR	NR	0.9
BLCA	411	2.7	3.4	48.4	0.2	NR	1.5	1.2	2.9	1.0	NR	0.5	1.0
LUSC	487	1.0	2.7	8.4	NR	NR	1.0	NR	15.8	NR	NR	0.3	NR
CESC	297	0.2	1.5	66.7	0.4	NR	NR	0.8	15.1	NR	NR	0.8	1.7
HNSC	523	NR	NR	91.6	2.1	NR	1.1	3.2	31.6	NR	NR	2.1	1.1
ESSCA	95	1.1	0.4	3.2	0.4	NR	0.7	NR	0.7	NR	NR	NR	NR

Table S3: DSC3 co-relation with other desmosomal components

Cancer	DSC1	DSC2	DSG1	DSG2	DSG3	DSG4	PKP1	PKP2	PKP3	JUP
KIPAN	0.1	0.2	0.3	0.2	0.3	0.2	0.4	0.2	0.0	0.0
LIHC	0.1	0.3	0.0	0.3	0.5	0.1	0.4	0.1	0.4	0.0
LUAD	0.3	0.4	0.2	0.3	0.5	0.2	0.1	0.3	0.0	0.1
PAAD	0.1	0.3	0.1	0.3	0.3	0.3	0.3	0.1	0.0	0.1
ESACA	0.5	0.6	0.5	0.0	0.7	0.3	0.8	0.0	0.4	0.2
OV	0.5	0.6	0.3	0.4	NA	0.3	0.2	0.0	-0.2	0.0
BRCA	0.6	0.5	0.8	0.4	0.9	0.2	0.6	0.2	0.0	-0.1
BLCA	0.4	0.5	0.4	0.3	0.7	0.2	0.6	0.1	0.5	0.3
LUSC	0.6	0.7	0.6	0.3	0.9	0.4	0.7	-0.2	0.4	0.6
CESC	0.7	0.8	0.8	0.0	0.9	0.6	0.8	-0.3	0.5	0.8
HNSC	0.5	0.8	0.6	0.2	0.9	0.5	0.6	0.1	0.3	0.5
ESSCA	0.6	0.7	0.5	0.2	0.8	0.5	0.6	0.0	0.5	0.7

Table S4A: Spearman correlation analysis with cancer hallmark genes

Sustained proliferative signalling (SPS)	Evading growth Suppressors (EGS)	Activating invasion and metastasis (AIM)	Enabling Replicative immortality (ERI)	Inducing angiogenesis (INA)	Resisting cell death (RCD)	Avoiding immune destruction (AID)	Tumour promoting Inflammation (TPI)	Genome instability and mutation (GIM)	Deregulating cellular energies (DCE)
AP1B1	TP53	CRABP2	RANGAP1	AGTR2	BAK1	ARG1	CD40	BRCA1	BRAF
ASXL1	TP63	BACH1	MCM5	ANG	BAX	CCR7	IL1A	BRCA2	HIF1A
BAD	TP73	PTTG1	MCM2	CD36	BCL2	CD27	IL1R1	ERCC1	HIF1AN
BAP1	MDM2	WASF3	CCND1	COL18A1	BCL2L1	CD274	IRAK3	ERCC2	HIF3A
BCOR	CDK2	CCL5	CDT1	CSF3	BCL2L2	CD276	NR3C1	ERCC3	IDH1
CAMTA1	RB1	CSF1	CDK6	CXCL12	BECN1	CD80	TNF	ERCC4	IDH2
CCNA1	STK11	PDIA6	CDK4	CXCL8	CASP3	CD86		ERCC5	KRAS
CCND1	NF2	EZR	DAXX	CXCR4	CASP8	CXCL9		ERCC6	LDHA
CDKN2A	TGFB1	PERP	ATRX	FGF2	FOXA1	CXCR6		ERCC8	PFKFB2
CDKN2B	TGFB2	SERPINB5	CCND2	MMP1	FOXL2	CYBB		HRNR	POGLUT1
CTNNB1	TGFB3	CDH1	AURKA	PDGFA	FOXO1	IDO1		MLH1	PRKAA1
GSTA1		CDH2	CCND3	TGFA	FOXO3	IL12B		MRE11	PRKAA2
HHEX		CDH3	CDKN2A	THBS1	FOXO4	IL1B		MSH2	
HMOX1		SNAI1	TERT	TIMP1	HIP1	IL27		PMS1	
JUN		SNAI2		TIMP2	HLF	IL4		PMS2	
LEF1		TWIST1		TIMP3	MEIS1	IL6		PMS2	
LRP1B		ZEB1		TIMP4	NAE1	LAG3		POLH	
MAPK1				VEGFA	NFIL3	MARCO		RAD50	
MEIS1				VEGFB	NFKB1	NKG7		RAD52	
MGLL				VEGFC	NFKB2	NOS2		RECQL4	
MLLT11				VEGFD	NODAL	PDCD1LG2		WRN	
MN1					PLAG1	PPARG		XPA	
MTOR					PML	SOCS3			
MYC					STAT5A	STAT1			
MYCL					TRAF7	STAT6			
NFE2L2					TRIM27	TIGIT			
NFKB1					WWTR1				
NODAL									
NOTCH1									
NOTCH2									
NOTCH3									
NOTCH4									
NQO1									
NR4A3									

PIK3CA									
PRKCA									
RAC1									
RBM10									
SF3B1									
SND1									
SOCS1									
STAT3									
STAT5A									
TBX2									
TET2									
TRAF1									
TRAF2									
TRAF3									
TRAF3IP1									
TRAF3IP2									
TRAF3IP3									
TRAF4									
TRAF5									
TRAF6									
TRAF7									
TRAFD1									
WT1									
WWTR1									
YAP1									

Table S4B: Correlated cancer hallmark genes (spearman value ≥ 0.5) with DSC3

Hallmark	Correlated Gene	KIPAN	LIHC	LUAD	PAAD	ESACA	OV	BRCA	BLCA	LUSC	CESC	HNSC	ESSCA
SPS	MYCL	0.1	-0.1	-0.1	0.2	0.4	0.0	0.3	-0.3	0.3	0.5	0.3	0.4
SPS	NFE2L2	0.1	0.0	0.1	0.0	0.3	-0.1	0.2	0.2	0.5	0.5	0.2	0.2
SPS	NOTCH1	0.2	0.3	0.1	0.0	0.2	0.1	0.5	0.3	0.3	0.6	0.1	0.2
SPS	NOTCH3	0.1	0.2	0.1	0.2	0.3	0.1	0.3	0.3	0.4	0.6	0.4	0.4
SPS	RAC1	0.0	0.0	0.2	0.1	0.3	-0.1	-0.1	0.3	0.2	0.5	0.1	0.2
SPS	PRKCA	0.1	0.0	0.2	0.0	-0.2	0.1	0.5	-0.1	-0.1	-0.3	0.0	0.1
SPS	TBX2	0.0	0.2	0.1	0.0	-0.1	0.0	0.0	-0.5	0.0	-0.3	-0.2	0.0
SPS	TRAF4	-0.1	0.0	-0.1	0.0	-0.1	0.0	0.0	-0.4	-0.2	-0.5	-0.3	-0.2
SPS	TRAF5	0.1	0.2	0.0	0.1	-0.2	0.0	-0.1	-0.2	-0.4	-0.5	-0.3	-0.4
EGS	TP63	0.2	0.2	0.3	0.5	0.7	0.1	0.6	0.4	0.6	0.8	0.4	0.4
AIM	PDIA6	0.1	0.0	-0.1	0.1	0.0	0.0	0.1	0.1	-0.1	-0.5	-0.1	0.2
AIM	PERP	0.0	0.0	0.2	0.2	0.5	0.1	0.4	0.6	0.6	0.8	0.5	0.6
AIM	SERPINB5	0.4	0.3	0.3	0.2	0.4	0.2	0.8	0.6	0.7	0.7	0.6	0.6
AIM	CDH1	0.1	0.1	0.1	0.1	0.2	0.1	-0.1	-0.1	0.2	0.1	0.5	0.5
AIM	CDH3	0.2	0.4	0.3	0.3	0.3	0.1	0.6	0.5	0.2	0.4	0.4	0.4
AIM	SNAI2	0.2	0.1	0.3	0.4	0.4	0.3	0.4	0.5	0.5	0.6	0.2	0.2
ERI	CDK6	0.2	0.1	0.2	0.2	0.0	0.1	0.4	0.5	0.2	0.2	0.2	0.3
INA	TIMP1	0.0	0.1	0.1	-0.1	0.0	-0.1	0.0	-0.1	-0.3	-0.6	-0.4	-0.4
RCD	BCL2L2	0.1	0.1	0.0	-0.1	0.1	0.1	0.0	0.2	0.4	0.5	0.2	0.2
TPI	IL1A	-0.1	0.2	0.2	0.0	0.5	0.1	0.2	0.1	0.2	0.5	0.1	0.3

Discussion:

Higher than normal expression of DSC3 seen in pancreatic, bladder, squamous esophageal, cervical squamous cancer and squamous lung cancer (Table 1) correlates with DSC3 protein seen in these patients (Valentina *et al.,*2009, Uhlen *et al.,* 2010, Uhlen *et al.,* 2017, Robertson *et al.,*2017, Wang *et al.,*2014,). Epigenetic silencing can be the reason for discordance between molecular expression and protein expression as seen in breast cancer (Oshiro *et al.,*2005).

Mutations of p53 are seen in majority of cancers and is a known major driver mutation (Bailey *et al.,*2018). In spite of being p53 dependent gene, DSC3 gene mutations were very low across various cancers studied indicating presence of different regulatory mechanism.

Better survival is seen in cancers with high DSC3expression (Group-III) and poor survival in low DSC3 expression (Group-I) (Table 2) in this study is in line with previous findings of better survival in LUSC (high DSC3 expression; Group III) (Belani *et al.,*2017, Cui *et al.,*2012) and poor survival in ovarian cancer (low DSC3 expression ; Group I) (Salerno *et al.,*2016). The correlation of survival with DSC3 expression level seen this study may explain the difference seen LUSC and ovarian cancer, and also suggest to consider DSC3 expression while evaluating DSC3 as a prognostic parameter in future studies.

Desmosomal integrity is prognostic of better outcome (Jodi *et al.,*2014). No correlation was seen between DSC3 expression level and desmosomal components in group I cancers and variable correlation was seen between DSC3 and desmosomal component seen in group II and III cancers. This is indicative of loss of desmosome integrity in this group I and may have contributed to poor survival seen in this group while using DSC3 as a prognostic parameter. High correlation of DSC3 with DSG3 (Fig. 1) in group II and III of this study could be due to their adjacent location on chromosome 18 (Berika & Garrod 2014). Correlation of DSC1, DSG1, PKP1 with DSC3 seen in this study is a novel finding (Fig. 1, Table S3). DSC3 as well as DSG3 expression is described to have reciprocal relationship with DSC1, DSG1 and PKP1 in normal as well as cancers (Jodi *et al.,*2014, Berika & Garrod 2014, Ferone *et al.,*2013, Khan *et al.,*2006, Green *etal.,*2019, Dusek & Attardi 2011). Initially DSG1 and DSC1 are expressed in basal layers and then migrate to superficial layers (Berika & Garrod 2014, Ferone *et al.,*2013, Khan *et al.,*2006, Green *etal.,*2019). It appears that they are retained in basal proliferative layers in along with DSC3 in group II and III cancers. PKP1 is known for growth control and hyper adhesion (Ferone *et al.,*2013, Khan *et al.,*2006, Green *etal.,*2019, Dusek & Attardi 2011). Correlation of PKP1 with DSC3 in this group might have contributed to better survival.

DSC3 expression was found to be correlated with various genes representing various hallmark in Group II and III (higher DSC3) and with none in group I. (Fig. 2, Table S4B). Correlation between DSC3 and Group II and III cancer was seen for EGS and AIM hallmark. Genes maximally correlated

with DSC3 across both groups are SERPINB5 (AIM), PERP (AIM), TP63 (EGS), and SNAI2 (AIM) (Fig. 2, Table S4B). Like DSC3, SERPINB5 and PERP are desmosomal proteins and their correlation with DSC3 suggest desmosomal integrity (Dusek & Attardi 2011). DSC3, PERP, SERPINB5, and SNAI2 gene expression is known to be TP63 dependent (Ferone *et al.,*2013, Khan *et al.,*2006). DSC1, DSG1, DSG3 are other desmosomal genes which are TP63 regulated and are correlated with DSC3 expression in group II and III. Increase in p63 is reported in squamous cancer of lung, head - neck, esophagel cancer, cervical cancer, bladder cancer (Moses *et al.,*2019). This indicate the significance of TP63 in DSC3 expressing cancers. DSC3 is a known master regulator as well as tumour suppressor (Moses *et al.,*2019, Ihrie *et al.,*2005).

Of TP63 isoforms, ΔNP63 is described to be associated with DSC3 in various disorder (Koster *et al.,*2014). ΔNP63 is also known oncogene which promotes cancer development but controls invasion and metastasis (Srivastava *et al.,*2018). DSC3, PKP1, Maspin and PERP (Green *et al.,*2019) are known for tumour suppression and inhibition of tumour invasion and metastasis (Jodi *et al.,*2014, Dzinic *et al.,* 2015, Bodenstine *et al.,*2012, Lockett *et al.,*2005). All these factors might have contributed to correlation between DSC3 expression and better survival.

Epithelial mesenchymal transition (EMT) is associated with appearance of CDH3 and disappearance of CDH1, (Dusek *et al.,* 2011). both belonging to AIM hallmark as well as adherence junction. Like CDH3, SNAI2 (AIM) is also associated with EMT and also has a reciprocal relationship with CDH1. In this study correlation of DSC3 with CDH1 expression and SNAI2 expression were mutually exclusive. HNSC and ESSCA expressed CDH1 and CESC, LUSC expressed SANI2. Relationship between CDH1 and desmosomal proteins is well known (Dusek *et al.,* 2011). but the correlation of mesenchymal marker CDH3 and SNAI2 with DSC3 an epithelial marker seems to be unique and is indicative of proposed EM3 stage within EMT spectrum (Nieto *et al.,*2016) in group I and II.

Present study suggests, DSC3 expression level may be useful for prognostication. It also suggests critical role of TP63 regulated desmosomal components in DSC3 expressing cancers. Association of DSG1, DSC1, PKP1, and SNAI2 with DSC3 in group II and III cancers is indicative of different biological process in these cancers. This study utilised TCGA dataset for DSC3 gene expression and its correlation. In all cancers studied, no information about epigenetic changes and DSC3 protein expression was available and so could not be studied. Epigenetic silencing of DSC3 gene is well known (Babicki *et al.,* 2016, J. Pan *et al.,* 2014). This needs to be evaluated for better prognostication in future studies. Correlation between DSC1, DSG1, PKP1 and DSC3 seen in this study is indicative of different biological process which needs further exploration.

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