INTRODUCTION

The gene **Disabled-2 (Dab2)** which also names DOC-2 (Differentially expressed in the Ovarian Carcinoma 2, DOC-2) owns two different splicing formats, and encodes two isoforms (p96-Dab2 and p67-Dab2). The main functional domain is the phosphotyrosine binding domain (PTB) of the N-terminal, which is a highly conserved sequence and plays a variety of functional roles in endocytosis, cell mitosis, and growth factor signaling. Especially, the p96-Dab2 is essential for the development of visceral endoderm during mouse embryogenesis and homologous with 93% full-length of mouse Dab2. Mechanistically, Dab2 is shown to bind with the growth factor receptor binding protein 2 (Grb2), consequently uncouple the activation of *c-Fos* expression and Ras/mitogen activated protein kinase (MAPK).

Accumulated studies have shown that Dab2 is reduced or lost in human cancers, containing lung cancers, nasopharyngeal carcinomas, the breast cancers, and colorectal cancers, et al. Thus, it is gradually considered as a tumor suppressor gene. However, there are no complete credible studies to explain the concrete mechanisms, except a few of epigenetic studies about promoters or exons of **Dab2**.

ABSTRACT

**Objective:** Disabled-2 (Dab2) is an important endocytic adaptor which plays an inhibition role in cancer cell growth. The objective of this study was to systematically review expressions of Dab2 in human cancers.

**Methods:** Eligible studies about Dab2 in human cancers were retrieved from databases of PubMed, Embase, Web of Science. Odds Ratios (ORs) with 95% confidence intervals (CIs) were calculated using Review Manager 5.0 software and statistical analyses were performed by the SPSS 13.0 software.

**Results:** Fourteen case-control studies with a total of 689 human tumor tissues, 332 control tissues and 32 cancer cell lines were included in the meta-analysis study. The results indicated loss expressions of Dab2 were observed in 74.9% and 46.9% in human malignant cancer tissues and cancer cell lines, respectively. The ratio of *Dab2* promotor hypermethylation is 34.54% in cancer tissues which Dab2 expression are lost, but none in the control tissues or cells by Methylation-specific PCR (MSP).

**Conclusions:** The expressions of Dab2 are frequently lost in human malignant cancer tissues, and promotor hypermethylation of Dab2 are common in human malignant cancer tissues, which is an important factor for the loss expression of Dab2 in human cancers tissues.

**KEY WORDS:** Dab2; Expression; Human; Cancers; Meta-Analysis.

doi: http://dx.doi.org/10.12669/pjms.302.4486

How to cite this:
Zhang Z, Chen Y, Tang JJ, Xie X. Frequent loss expression of dab2 and promotor hypermethylation in human cancers: A meta-analysis and systematic review. Pak J Med Sci 2014;30(2):432-437. doi: http://dx.doi.org/10.12669/pjms.302.4486

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
In the current study, we firstly conducted a meta-analysis of cohort studies to evaluate Dab2 expression level and its epigenetic variations in various human malignant cancers or cell lines. Furthermore, systematically investigated the concrete reasons for Dab2 expression loss, and its correlation with human oncogenesis.

METHODS

**Literature search strategy:** We adapted the Cochrane Central Register of Controlled Trials, and searched relevant papers published before September 31st 2013 in Medline, Embase, Web of Science, and Scopus with relevant text words and medical subject headings that included all spellings of “Disabled-2” or “DOC-2” and “cancer” and “human”. In addition, we firstly performed an initial scanning of the titles or abstracts, reference lists of retrieved papers and reviews to identify other potential relevant studies. Disputes were resolved through discussions among three authors.

**Criteria of Inclusion and Exclusion:** We selected the eligible studies in the present meta-analysis using the following criterions: (1) use of an cohort study or case-control study design, and focusing on the correlations between Dab2 or DOC-2 and human cancers; (2) basic researches with big size of tissue samples. Studies were excluded using the following criterions: (1) not a case-control study or cohort study; (2) not a primary document, such as a letter, meta-analysis, review, duplicate or editorial article; (3) literatures with insufficient samples or incomplete data, and the conclusions are out of date.

**Data extraction:** Three authors recorded the following details of each included research cooperatively, containing: authors, year of publication, the country of study, journal, materials and methods, study design, age of study population, pathological type of tumors, detecting sample size, source of participants, confounders adjusted for, effect sizes and 95% Confidence Index (CI) or standard errors of effect sizes. Differences were resolved by discussion among three authors in cases of conflicting evaluations.

**Statistical Analysis:** The present case-control study was performed by Review Manager 5.0 software, the direct count method was used to estimate the expression level of Dab2 in human cancers. The odds ratios (OR) and 95% confidence intervals (CI) were computed by means of the Fisher’s exact probability test (two-tailed p-values). The data was analyzed by means of SPSS version 13.0 (SPSS Inc; Chicago, USA). This merged ORs and the 95% CI were obtained by means of the fixed or random effects model for each kind of human cancers. The heterogeneity was computed by Cochrans Q test, which P-value > 0.05 or I^2 > 50% indicated the existence of heterogeneity among studies. The subgroup analysis was used to explore sources of heterogeneity.

**RESULTS**

**Search of Eligible Studies:** We initially retrieved 112 relevant papers in September, 2013. Finally, 15 studies were included in the present meta-analysis.

**Characteristics of Included Studies:** The main characteristics of the 15 studies are presented in Table-I in which publication year ranges from 1998 to 2013^{12-27}, and the quality scores vary from 5.5 to 7.5 points. A total of 395 different human cancer tissues and 32 cancer cell lines were utilized to analyze the expression loss of Dab2 by immunohistochemistry or western blot analysis. Furthermore, the analysis of aberrant hypermethylation of Dab2 promoter were performed in Nasopharyngeal carcinomas, Esophageal Squamous Cell Cancers, breast cancers and lung cancers, respectively.

**Frequent loss expression of Dab2 was observed in various human cancers tissues:** The immunostained percentage of Dab2 expression were detected in five different kinds of human cancer tissues. Mok SC et al found that Dab2 expression were significantly
down-regulated in ovarian cancers than normal ovarian tissues (the strong positive rate was 4.5% Versus 84.1%, and weak positive rate was 92.3% versus 0.0%). Analogously, Xu et al reported that Dab2 expression was significantly reduced in lung cancers than the non-cancerous tissues (the strong, moderate, and weak positive rate were 56.2% Versus 24.76%, 37.1% Versus 47.62% and 6.7% Versus 27.62%, respectively) Table-II. Furthermore, Tong et al found that Dab2 was un-detectable in 72% nasopharyngeal carcinomas of Chinese people.

Table-I: Characteristics of eligible studies for Dab2 expression in human cancers.

| Country | The First Author Year | Materials | Methods | QS |
|---------|-----------------------|-----------|---------|----|
| USA     | SC Mok 1998 [12]      | 44 ovarian cancers, 16 borderline ovarian tumors, 6 benign ovarian tumors, 13 normal human ovaries | IHC | 6.5 |
|         | Vilmos Fulop 1998 [20] | 17 partial hydatidiform moles, 25 complete moles and 11 gestational choriocarcinomas | IHC | 6.0 |
|         | Elizabeth R. Smith 2001 [21] | F9 mouse and PA-1 (human teratocarcinoma cells) | WB | 5.5 |
|         | Zj Sheng 2000 [23]     | 47 paired ovarian tumor and non-tumor tissues | IHC | 5.5 |
|         | Shao-Chun Wang 2001 [24] | 2 breast cancer cell lines | IHC, WB | 5.5 |
|         | DH Yang 2002 [13]      | 50 ovarian tumor and 5 non-tumor tissues | IHC | 6.5 |
|         | Jian Zhou 2005 [25]    | Seven human transitional cell carcinoma (TCC) cell lines | WB | 6.0 |
|         | Jian Zhou 2005 [26]    | 7 prostatic cancer cells c, 5 normal prostatic epithelial cells d, and PZ-HPV7 cell | WB | 6.5 |
|         | JoseA Karam 2007 [21]  | 209 patients with Malignant urothelial Carcinoma of the Bladder, 9 patients with normal blader, 44 patients with Ta, Tis, or T1 UCB | IHC | 7.0 |
| China   | Hong-Tao Xu 2011 [15] | 105 lung cancer and 105 matched normal tissue samples | IHC | 7.0 |
|         | Xue-Mei Xie 2013 [14] | 100 lung cancer and paired normal tissue samples, eight lung cancer cell lines | WB, MSP | 7.5 |
|         | Joanna H Tong 2010 [16] | 3 NPC cell lines f, 5 xenografts g, 46 nasopharyngeal carcinoma tissue samples. | WB, MSP | 7.5 |
| India   | S. A. R. Bagadi 2007 [17] | 6 breast cancer cell lines e, and MCF 10A, 54 breast cancer tissue samples | WB, MSP | 7.5 |
|         | Kumar Anupam 2006 [19] | 50 ESCCs, 10 non-malignant esophageal mucosa, 30 hyperplasia and 15 dysplasia tissues | WB, MSP | 7.5 |
|         | Joanna Xie 2013 [14]   | 100 lung cancer and paired normal tissue samples, eight lung cancer cell lines | WB, MSP | 7.5 |
| Korea   | Seong-Moon Cheong 2012 [27] | 8 cancer cell lines h and 1 human umbilical vein endothelial cell | WB | 7.0 |

QS: Quality Score; IHC: Immunohistochemistry; WB: Western Blot analysis.
a SK-BR-3 and MDA-MB-453 cell lines. b T24, TCC, UMUC3, WH, SWB1C2, 253J, and RT4 cell lines.
c LNCaP, C4-2, p59-23 clone(13), COS cells; LAPC4, MDAPCa2a, and MDA-PCa2b cell lines.
d PrEC1, PrEC2, PrEC3, SWNPC2, SWPC1, and SWPC3 cell lines.
e C666-1, HK1 and HONE1. g X2117, X666, C15, C17, and X1915.
f A549 (lung cancer), SH-SY5Y (neuroblastoma), MDA-MB231 and MCF7 (breast cancer), HT1080 (fibrosarcoma), HepG2 (hepatoma), Du145 (prostate cancer), and SW480 (colon cancer).

The ectopic expressions of Dab2 were observed in several human malignant cancer cell lines: Data pooled from eight studies in this meta-analysis showed that the ectopic expression of Dab2 were observed in 17 human malignant cancer cell lines, including: A549, LTE, H1299, SH-SY5Y, HT1080, et al. Dab2 was un-detectable in MCF7, T47D, ZR-75-1,
Du145, et al.14,16,17,22,24-27 There was no significant difference between weak positive and absent expression of Dab2 in human cancer cells (OR = 1.04, 95% CI: 0.66 - 1.65, $P = 0.85$; $I^2 = 39.9\%$, $P_{\text{heterogeneity}} = 0.11$) (Fig. 2B). Results of the Pearson $\chi^2$ test revealed that abnormal expression of Dab2 was not significant correlated with the types of cancers from which cancer cell lines originated ($\chi^2 = 3.23$, $P = 0.36$).

Table-II: The expression of Dab2 in five kinds of human cancerous and the corresponding Non-Cancerous tissues by immunohistochemistry.

| Type of cancers* Tissues | Expression level of Dab2 | Total |
|-------------------------|--------------------------|-------|
|                         | High | Moderate | Weak or Negative |

| LC Lung Tissues          | Non-Cancerous | Count | 59 | 39 | 7 | 105 |
|                         | % within Tissues | 56.2% | 37.1% | 6.7% | 100.0% |
|                         | Malignant Cancerous | Count | 29 | 50 | 26 | 105 |
|                         | % within Tissues | 27.6% | 47.6% | 24.8% | 100.0% |

| UCB Bladder Tissues      | Non-Cancerous | Count | 8 | 1 | 0 | 9 |
|                         | % within Tissues | 88.9% | 11.1% | 0.0% | 100.0% |
|                         | LP or Benign Tumors | Count | 18 | 26 | 0 | 44 |
|                         | % within Tissues | 40.9% | 59.1% | 0.0% | 100.0% |
|                         | Malignant Cancerous | Count | 52 | 117 | 40 | 209 |
|                         | % within Tissues | 24.8% | 56.0% | 19.2% | 100.0% |

| GC Trophoblast-ic Tissues | Non-Cancerous | Count | 14 | 4 | 0 | 18 |
|                         | % within Tissues | 77.8% | 22.2% | 0.0% | 100.0% |
|                         | LP or Benign Tumors | Count | 8 | 15 | 19 | 42 |
|                         | % within Tissues | 19.0% | 35.7% | 45.2% | 100.0% |
|                         | Malignant Cancerous | Count | 0 | 4 | 7 | 11 |
|                         | % within Tissues | 0.0% | 36.4% | 63.6% | 100.0% |

| ESCCs Esophageal Tissues  | Non-Cancerous | Count | 30 | 5 | 5 | 40 |
|                         | % within Tissues | 75.0% | 12.5% | 12.5% | 100.0% |
|                         | LP or Benign Tumors | Count | 0 | 5 | 10 | 15 |
|                         | % within Tissues | 0.0% | 33.3% | 66.7% | 100.0% |
|                         | Malignant Cancerous | Count | 0 | 16 | 34 | 50 |
|                         | % within Tissues | 0.0% | 32.0% | 68.0% | 100.0% |

| HOTs Ovarian Tissues     | Non-Cancerous | Count | 12 | 1 | 0 | 13 |
|                         | % within Tissues | 92.3% | 7.7% | 0.0% | 100.0% |
|                         | LP or Benign Tumors | Count | 8 | 11 | 3 | 22 |
|                         | % within Tissues | 36.4% | 50.0% | 13.6% | 100.0% |
|                         | Malignant Cancerous | Count | 2 | 5 | 37 | 44 |
|                         | % within Tissues | 4.5% | 11.4% | 84.1% | 100.0% |

LC: Lung Cancers; UCB: Urothelial Carcinoma of the Bladder; GC: Gestational Choriocarcinomas; ESCCs: Esophageal Squamous Cell Cancers; HOTs: Human Ovarian Tumors. LP: Lesion Precancerous.
Fig. 3: Forest plots of the meta-analysis of promoter hypermethylation of Dab2 in human cancers tissues and cell lines.

**Reduced expression of Dab2 was correlated with the aberrant promoter hypermethylation in human cancers:** Bisulfite sequencing and methylation specific PCR (MSP) were employed to explore the correlations between promoter aberrant hypermethylation of Dab2 and expressing reduction in 4 studies.\(^{16,17,19}\) Subgroup analysis was applied to discriminate the discrepancies of aberrant promoter hypermethylation of Dab2 in cancer tissues and cell lines. Results showed that ratio of Dab2 promoter hypermethylation is 34.54\% in cancer tissues which Dab2 expression are lost, and Dab2 promoter hypermethylation might play a key role in the down-regulated expression of Dab2 in human cancer tissues (OR = 24.45, 95\% CI: 11.00 - 54.32, P < 0.001; \(I^2 = 45\%\), \(P_{\text{heterogeneity}} = 0.14\)) (Fig. 3).

**Publication bias:** Egger test\(^{28}\) was performed to observe potential publication bias in each meta-analysis, and results showed no evidence of publication bias for each outcome: expression loss in human cancers tissues \(P_{\text{Egger}} = 0.092\), expression reduced in various cancer cell lines \(P_{\text{Egger}} = 0.086\) and aberrant promoter hypermethylation \(P_{\text{Egger}} = 0.061\).

**DISCUSSION**

In this first systematic review, Dab2 expression was analyzed in approximately 789 human tumor and 432 normal tissues of 15 included papers. Some studies demonstrated that Dab2 protein was un-detectable in 70\% ~ 90\% human malignant cancers, including nasopharyngeal carcinomas, breast cancers, and gestational choriocarcinomas.\(^{16,17,20}\) However, other studies have suggested the weak to moderate positive immunostained of Dab2 expression in lung cancers, and ESCCs, et al.\(^{15,19,21}\) These diversities may be correlated with the tissue-specific differentially expression patterns of Dab2.\(^{10,11}\) Similarly, our previous studies on lung cancers suggest that there are different functions between p96-Dab2 and p67-Dab2 in the process of oncogenesis.\(^{14}\)

Unfortunately, some weaknesses of current researches on Dab2 in cancer cell lines are identified in this meta study. Bagadi et al. reported that Dab2 was lost in all the breast cancer cell lines containing MDA-MB-231\(^{17}\); conversely, Cheong et al. hold opinions that it was weak positive not absent expression of Dab2 in MDA-MB-231.\(^{27}\) More interestingly, we and other researchers found that both lung cancer and 60\% of TCC cell lines showed week to moderate positive expression of Dab2 protein.\(^{14,25}\) Thus, there is not enough evidence to determine that whether the loss or weak positive of Dab2 expressions are analogous in all the human malignant cancer cell lines \(P = 0.85\). Furthermore, new standards must be ruled out for the evaluation of Dab2 expression in cancer cell lines in the near future.

Our research reveals that the promoter hypermethylation of Dab2 is an important factors for the loss expression of Dab2 in human cancers tissues (OR = 24.45, \(P < 0.001\)). Although Dab2 promoter hypermethylation have been observed in some cancer cells, there are still few reasons to attribute down-regulated expression of Dab2 to the promoter hypermethylation unless further credible evidences emerge from other cancer cells \(P = 0.19\).

In conclusion, frequent loss expressions of Dab2 are common in human malignant cancer tissues, and significantly correlated with the promoter hypermethylation. More studies would be conducted to enhance the expression of Dab2, and eliminate the aberrant hypermethylation of Dab2, which would offer some potential therapeutic treatment methods for human malignant cancers.

**Conflict of interest:** We declare that we have no conflict of interest.

**REFERENCES**

1. Mok SC, Wong KK, Chan RK, Lau CC, Tsao SW, Knapp RC, et al. Molecular cloning of differentially expressed genes in human epithelial ovarian cancer. Gynecol Oncol. 1994;52(2):247-252.

2. Xu XX, Yang W, Jackowski S, Rock CO. Cloning of a novel Phosphoprotein regulated by colony-stimulating factor 1 shares a domain with the Drosophila disabled gene product. J Biol Chem. 1995;270(23):14184-14191.
Frequent loss expression of dab2 and promoter hypermethylation in human cancers

Author Contributions:

ZyZ conceived, designed and did statistical analysis & editing of manuscript.
YhC, XmX, & JjT did data collection and manuscript writing.
XmX did review and final approval of manuscript.
XmX was responsible for planning the study.

References:

1. Madden DR, Swiatecka-Urban A. Tissue-specific control of CFTR endocytosis by Dab2: Cargo recruitment as a therapeutically targeted. Commun Integr Biol. 2012;5(5):473-476. doi: 10.4161/cib.21375.

2. Fu L, Rab A, Tang LP, Rowe SM, Bebok Z, Collawn JF. Dab2 is a key regulator of endocytosis and post-endocytic trafficking of the cystic fibrosis transmembrane conductance regulator. Biochem J. 2012;441(2):633-643. doi: 10.1042/ BJ20111566.

3. Zeng X, Tamak M, Doble B, Li S, Huang H, Habas R, et al. A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. Nature. 2005;438(7069):873-877.

4. Sheng Z, Smith ER, He J, Tuppen JA, Martin WD, Dong FB, et al. Chromosomal location of murine disabled-2 gene and structural comparison with its human ortholog. Gene. 2001;268(1-2):31-39.

5. Kim JA, Bae SH, Choi YJ, Kim KH, Park SS. Feed-back regulation of disabled-2 (Dab2) p96 isoform for GATA-4 during differentiation of F9 cells. Biochem Biophys Res Commun. 2012;421(3):591-598. doi: 10.1016/j.bbrc.2012.04.051.

6. Zhou J, Scholes J, Hsieh JT. Characterization of a novel negative regulator (DOC-2/DAB2) of c-Src in normal prostatic epithelial and cancer. J Biol Chem. 2003;278(9):6936-6941.

7. Zhou J, Fan J, Hsieh JT. Inhibition of mitogen elicited signal transduction and growth in prostate cancer with a small peptide derived from the functional domain of DOC-2/DAB2 delivered by a unique vehicle. Cancer Res. 2006;66(18):8954-8958.

8. Mok SC, Chan WY, Wong KK, Cheung KK, Lau CC, Ng SW, et al. DOC-2, a candidate tumor suppressor gene in human epithelial ovarian cancer. Oncogene. 1998;16(18):2381-2387.

9. Wang SC, Makino K, Xia W, Kim JS, Im SA, Peng H, et al. DOC-2/hDab-2 inhibits ILK activity and induces anoikis in breast cancer cells through an Akt-independent pathway. Oncogene. 2001;20(47):6960-4.

10. Zhou J, Hernandez G, Tu SW, Scholes J, Chen H, Tseng CP, et al. Synergistic Induction of DOC-2/DAB2 Gene Expression in Transitional Cell Carcinoma in the Presence of GATA6 and Histone Deacetylase Inhibitor. Cancer Res. 2005;65(14):6089-6096.

11. Zhou J, Hernandez G, Tu SW, Huang CL, Tseng CP, Hsieh JT. The role of DOC-2/DAB2 in modulating androgen receptor-mediated cell growth via the non-genomic c-Src-mediated pathway in normal prostatic epithelium and cancer. Cancer Res. 2005;65(21):9906-9913.

12. Cheong SM, Choi H, Hong BS, Gho YS, Han JK. Dab2 is pivotal for endothelial cell migration by mediating VEGF expression in cancer cells. Exp Cell Res. 2012;318(5):550-557.

13. Mok SC, Chan WY, Wong KK, Cheung KK, Lau CC, Ng SW, et al. DOC-2, a candidate tumor suppressor gene in human epithelial ovarian cancer. Oncogene. 1998;16(18):2381-2387.

14. Yang DH, Smith ER, Cohen C, Wu H, Patriots C, Godwin AK, et al. Molecular events associated with dysplastic morphologic transformation and initiation of ovarian tumorigenesis. Cancer. 2002;94(9):2380-2392.

15. Xie XM, Zhang ZY, Yang LH, Yang DL, Tang N, Zhao HY, et al. Aberrant hypermethylation and reduced expression of disabled-2 promote the development of lung cancers. Int J Oncol. 2013;43(3):1636-1642. doi: 10.3892/ijo.2013.2084.

16. Xu HT, Yang LH, Li QC, Liu SL, Liu D, Xie XM, et al. Disabled-2 and Axin are concurrently co-localized and under-expressed in lung cancers. Hum Pathol. 2011;42(10):1491-1498. doi: 10.1016/j.humpath.2011.01.004.

17. Tong JH, Ng DC, Chau SL, So KK, Leung PP, Lee TL, et al. Putative tumour-suppressor gene DAB2 is frequently down regulated by promoter hypermethylation in nasopharyngeal carcinoma. BMC Cancer. 2010;10:253. doi: 10.1186/1471-2407-10-253.

18. Bagadi SA, Prasad CP, Srivastava A, Prashad R, Gupta SD, Ralhan R. Frequent loss of Dab2 protein and infrequent promoter hypermethylation in breast cancer. Breast Cancer Res Treat. 2007;104(3):277-286.