# Expression of Death Associated Proteins DAP1 and DAP3 in human pancreatic cancer

Laijian Sui[1,2], Lin Ye[1], Andrew J. Sanders[1], Yiming Yang[1], Chunyi Hao[3], Rachel Hargest[1] and Wen G. Jiang[1]

[1]Cardiff China Medical Research Collaborative, Cardiff University School of Medicine, Cardiff, United Kingdom

[2]Yantai Yuhuangding Hospital, Shandong, People's Republic of China

[3] Key laboratory of Carcinogenesis and Translational Research (Ministry of Education, Beijing), Department of Hepato-Pancreato-Biliary Surgery, Peking University Cancer Hospital & Institute, Beijing, 100142, China

**Correspondence to**: Professor Wen G. Jiang, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, UK. Tel: 00 44 29 2068 7065, email: jiangw@cardiff.ac.uk.

**Running title**: Death associated proteins in pancreatic cancer

**Key words**: DAP3, DAP1, Death Associated Proteins, pancreatic cancer, survival, prognosis

**Abstract.** Background: Death associated proteins (DAPs) are involved in the apoptosis of various cell types in response to interferon gamma, including cancer cells. The present study assessed both DAP1 and DAP3 in human pancreatic cancer. Materials and Methods: DAP1 and DAP3 transcripts were quantitatively analysed in pancreatic tumour tissues and paired adjacent normal tissues using real time PCR followed by statistical analyses for their clinical implications. Results: Levels of DAP3 transcripts in pancreatic cancer were markedly higher than in normal tissues, whereas DAP1 had lower levels in cancer *versus* normal tissues. Adenocarcinomas showed higher levels of DAP3 than other histological types. Patients with high levels of DAP3 had a significantly shorter overall survival than those with low levels ($p$=0.012). The status of DAP3 and lymph node involvement identified patients with poor survival ($p$<0.00001). Conclusion: DAP3 was highly expressed in pancreatic tumour tissues and was significantly associated with shorter survival.

Death associated proteins (DAPs) are a small group of proteins that have been implicated in the programmed death process of various cell types including cancer cells. There are two known members, DAP1 (or DAP) and DAP3. DAP was initially discovered to be a factor in interferon-gamma (IFNγ) induced apoptosis in HeLa cells and is a 15kDa protein (1). The other member, DAP3, was discovered in the same year, again as an IFNγ inducible cell death related gene in the same cell type, encoding a 46kDa protein (2). DAP1 has since been identified as a substrate of the mammalian target of rapamycin (mTOR) and is a negative regulator of autophagy (3); it is a responsive gene to type II protein arginine methyltransferase PRMT5 in various cell types (4). DAP3 has also been shown to be a regulator of *anoikis*, cell adhesion dependent apoptosis in HEK293 cells (5).

DAPs have been linked to cell death and cell migration. DAP1 has been reported to be reduced or lost in glioblastoma and medulloblastoma cells (6). In contrast, DAP3 was found to be overexpressed in glioblastoma multiforme and knocking down DAP3 in glioblastoma cells results in a more mobile phenotype (7). Loss of DAP1 by knockdown resulted in cells with rapid growth and migration (8) in breast cancer. However, the opposite was seen in the ovarian cancer cell line SKOV3; knockdown of DAP1 reduced cell growth (9).

Since their discovery, there have been continued studies to explore the role of DAP1 and DAP3 in cancer cells and in some of the solid cancers. In human cancers, expression of DAP1 has been found to be reduced or lost in late stage tumours and tumours with recurrence or metastasis in breast cancer (10), and the reduction in colorectal cancer was associated with shorter survival of the patients (11). In contrast, higher levels of DAP1 have been reported in oral squamous cell carcinoma, in thyroid cancer (12), and in patients with lymph node metastasis of oral squamous cell carcinoma (13). In addition, DAP3 has been shown to be present at higher levels in glioblastoma multiforme tumours and in late stage thymomas (7, 14). In breast cancer, DAP3 expression was found to be reduced compared to normal tissues. Reduced DAP3 was seen in aggressive and late stage tumours and in patients who developed metastasis and recurrence (15). The same trends were observed in gastric cancer (16, 17). Reduced expression of DAP3 in patients with gastric cancer also resulted in chemoresistance (16). DAP3 is also linked to resistance to radiotherapies in lung cancer (18). In non-epithelial derived osteosarcoma cells, high levels of DAP3 have been reported to induce apoptosis of the tumour cells (19). Despite its importance in cancers, no significant mutations of the DAP3 gene, at least in its P-loop region, have been reported in multiple cancer tissues including gastric, liver, colorectal and lung (20).

Collectively, DAP1 and DAP3 have been shown to play important roles in the living and death of cancer cells and in the development and progression of certain solid tumours. However, their pro- or anti-apoptotic properties and their tumour suppressive or oncogenic properties remain unclear; they seem to primarily depend on the cell and cancer type.

Pancreatic cancer remains one of the most fatal malignancies worldwide accounting for 466,000 cancer deaths in 2020. The number of the patients who died from the this cancer type per year is almost the same as the number of newly diagnosed cases which was 496,000 for the same period (21). In contrast to the advances and improvement in the diagnosis and treatment of other malignancies, demand for early detection and effective therapeutic approaches for pancreatic cancer requires a better understanding of the corresponding molecular and cellular machinery. The present study investigated the expression profile of both DAP1 and DAP3 in a large cohort of human pancreatic cancer, together with a small public database. We report a clinical and survival benefit of reduced levels of DAP3 expression, and to a lesser degree DAP1, in patients with pancreatic cancer.

**Patients and Methods**

*Clinical cohort and collection of tumour tissues.* 223 patients entered the study. Tumour tissues and matched normal tissues were immediately collected after pancreatomy and stored in liquid nitrogen until further use. The study was supported by the Ethics Committee of Peking University Cancer Hospital and is in full accordance with the Helsinki declaration. Consents were obtained from the patients. Clinical and pathological information as well as follow up data were collected retrospectively. The median patient follow up was 12 months.

*Tissue processing and quantitative assessment of DAP1 and DAP3 gene transcript.* Tissues were homogenised in an RNA isolation buffer. RNAs were extracted, purified and quantified by a UV spectrophotometer. The RNA concentration was then standardised before reverse transcription was carried out. Reverse transcription was carried out using a reverse transcription kit (Promega, Southampton, UK). The primers used for DAP1 were ATGGACAAGCATCCTTCC and ACTGAACCTGACCGTACACTCTGTCAGGGAAATACCAA, for DAP3

AAAGCACTGAGAAAGGGAGT and
ACTGAACCTGACCGTACACCTCTTTAGCTCTTTCAGCA, and for GAPDH
AAGGTCATCCATGACAACTT and
ACTGAACCTGACCGTACAGCCATCCACAGTCTTCTG, in a 5 to 3' direction. The detection of amplicons was with a molecular beacon based Uniprimer$^{tm}$ system in which the FAM tagged Uniprimer$^{tm}$ worked with the reverse primer via a unique sequence, present in each of the reverse primers for the specific genes (the z sequence, underlined above). Quantitative PCR was carried out on a StepOne Plus qPCR detection system (Fisher Scientific, Loughborough, UK).

*Statistical analysis.* Pairwise comparisons were made using the Student's *t* test. Survival analysis was carried out with the Kaplan Meier's method. Multivariate analysis and logistic regression of clinical/pathological factors and levels of DAPs were carried out against clinical outcome. Comparison of number of deaths in the respective groups were carried out by Fisher Exact test. All the analyses were carried out using SPSS version 26 (IBM UK, Portsmouth, England, UK).

**Results**

*Expression of DAP1 and DAP3 in pancreatic cancer.* Expression levels of DAP1 and DAP3 in normal and tumour tissues showed a different pattern. DAP3 showed higher levels in tumour tissues whereas DAP1 showed lower levels in tumour tissues compared with normal tissues, but these differences did not reach statistical significance (Table I). Adenocarcinoma had significantly higher levels of DAP3 than and other histological types ($p$<0.0001). Overall, high levels of DAP3 are seem in node negative groups (p=0.14) but the same was not seen with DAP1 ($p$=0.75). There was no overall association between the degree of differentiation. Tumours from different anatomical location tend to have similar levels of DAP3, although tumours from the body (p=0.029) and tail (p=0.0021) region of pancreas had significantly lower levels of DAP1 than tumours from the head of pancreas (Table I). However, T-stage 3 and TNM2 tumours seem to have significantly higher levels of DAP1 ($p$=0.0021 and 0.01 *vs* stage 1and TNM1, respectively). Although other groups had higher levels than TNM1 and stage 1 tumours, the difference was not significant. A similar trend was seen with DAP3 but neither comparisons were significant.

*DAP3 and DAP1 were not correlated in pancreatic cancer tissue.* To explore if the two death associated proteins are indeed correlated in tissues in the pancreas, we undertook correlation

analysis for the two DAPs in the whole cohort, in normal and tumour tissues independently. Within the entire cohort, normal and tumour combined, a significant inverse correlation was seen between DAP3 and DAP1 ($r$=-0.097, $p$=0.019). However, when the tissues were separated into normal and tumour groups, no significant correlations were seen ($r$=-0.073, $p$=0.307 for tumour tissues and $r$=-0.054, $p$=0.388 for normal tissues). Overall, this indicates that the expression levels of DAP1 and DAP3 in pancreatic cancer tissues has little correlation.

*DAP3 and DAP1 expression and patient clinical outcome.* In our cohort, we found that there was a significant relationship between DAP3 and the patients' overall survival. Patients with high levels of DAP3 had significantly shorter survival compared to those with low levels (21.6±3.2 months *vs* 35.1±6.7 months respectively, $p$=0.012) (Figure 1A). In contrast, patients with high levels of DAP1 showed a marginally longer survival than those with lower levels (28.9±5.8 months *vs* 24.4±3.6 months respectively, $p$=0.74) (Figure 1C). This finding was supported by the TCGA dataset from a smaller cohort (n=178), where $p$=0.028 and 0.41 for DAP3 and DAP1, respectively (Figure-1B and 1D, respectively).

*DAP3 and patient clinical outcome stratified by DAP1.* We attempted to stratify the cohort by DAP1 expression and explore the value of DAP3 in patient survival. As shown in Figure 2, when the patients were segregated based on high and low DAP1 expression, DAP3 levels showed a greatly improved survival prognosis (Figure 2).

*Combined expression of DAP3 with nodal status dramatically enhances its value as independent prognostic survival indicator.* As shown in Table I, tumours with or without lymph node involvement had different levels of expression of DAP3. We cross analysed if lymph node status and DAP3 status had an impact on the survival of the patients. As seen in Table II, it is clear that patients who had high DAP3 and node positive tumours had the poorest outcome, with a survival rate at 5.8% during the follow up period, where the remain patients have significantly higher survival rate, namely 26.2% (p=0.0036), 34.8% (p=0.0013) and 32.0% (p=0.0022) for DAP3 high/node (-), DAP3 Low/Node (-) and DAP3 low/node (+), respectively. In this regard, the combination of DAP3 and nodal status can be a significant independent prognostic indicator for survival (HR 10.06, $p$=0.006) (Table III) and patients who were node positive and possessed high levels of DAP3 had poor rates of survival (13.6±2.8 months *vs* 29.4±3.7 months for the remaining patients, $p$<0.00001) (Figure 3).

**Discussion**

Although DAP proteins have been identified for their role in regulating apoptosis, *anoikis* and other cellular functions related to cancer cells including cell migration, DAP1 and DAP3 have been shown to play a tumour suppressive or stimulating role, depending on the cell and tumour types (7, 10-14). Clinically, the pattern of expression of DAP1 and DAP3 differs and it is sometimes contrasting within the same tumour type and dependent on the cancer histological type.

The present study, by employing a sizeable cohort of pancreatic cancers, has provided evidence that in pancreatic cancer, DAP1 and DAP3 also express a contrasting pattern in pancreatic cancers. This was seen in high levels of DAP3 but low levels of DAP1 in tumour tissues compared with normal tissues, high levels of DAP3 but low levels of DAP1 in adenocarcinoma compared with other histological malignancies of the pancreas and also contrasting patterns of DAP3 and DAP1 in tumours with microvessel embolism when compared with tumour without embolism. This clearly shows that in the same cohort and the same cancer type, the two DAPs have different and rather contrasting patterns. This contrast in the same tumour type is unexpected as both DAP1 and DAP3 were discovered as apoptosis related genes and we have anticipated a similar pattern of changes in the same tumour type.. The reasons underlying the contrast remain unclear. It is interesting to note that the two DAPs are located on different chromosomes, namely DAP1 on 5p15.2 and DAP3 to 1q22. It is highly plausible that the gene transcription regulations are very different. The contrasting patterns of expression of both DAPs seen here, and in the literature, strongly argue that their expression are indeed regulated differently or respond to environmental stimuli differently. Further work is required to fully understand these observations. Additionally, large scale study at protein levels, namely immunohistochemical analyses of both DAP1 and DAP3 would be important to evaluate if the differential pattern between the two molecules in pancreatic cancer and indeed other tumour types seen at protein level.

The most interesting finding of the study is the significant correlation between the levels of DAP3 expression and patient clinical outcome and that high levels of DAP3 are seen with significantly shorter survival. This correlation is in contrast to that of DAP1, in that high levels of DAP1 are seen with a marginally longer survival. DAP3 expression and survival in pancreatic cancer was similarly reported in glioblastoma multiforme tumours (7) and in late stage thymomas (14), but is opposite to those reported in breast (15) and gastric cancer (16, 17). Collectively, it is clear that DAP3 has prognostic value and is predictive of poor patient outcome. This relationship seems to be tumour type dependent.

DAP3 expression and lymph node involvement has been recently reported in squamous cell carcinoma of the oral cavity (13). The present study has also demonstrated that the status of DAP3 expression and lymph node involvement can together provide a powerful and independent indicator for patient survival. This collectively argues for a pivotal role of DAP3 in lymph node spread in pancreatic cancer and likely in other cancer types.

Overall, DAP3 and DAP1 have a contrasting expression pattern in human pancreatic cancer with DAP3 highly expressed in tumour tissues. Patients with high levels of DAP3 in their tumour tissues have significantly shortened survival indicating an independent prognostic value.

## Authors' Contributions

Experimental design: CH and WGJ; Data collection: LY, AJS, CH and WGJ; Data analysis: LS, YM, AJS, JY and WGJ; Manuscript preparation: all.


## Acknowledgements

This study was supported by Cancer Research Wales and Cardiff China Medical Scholarship.

## References


1 Deiss LP, Feinstein E, Berissi H, Cohen O and Kimchi A: Identification of a novel serine/threonine kinase and a novel 15-kd protein as potential mediators of the gamma interferon-induced cell death. Genes Dev 9(1): 15-30, 1995. PMID: 7828849 DOI: 10.1101/gad.9.1.15.

2 Kissil JL, Deiss LP, Bayewitch M, Raveh T, Khaspekov G and Kimchi A: Isolation of dap3, a novel mediator of interferon-gamma-induced cell death. J Biol Chem 270(46): 27932-27936, 1995. PMID: 7499268 DOI: 10.1074/jbc.270.46.27932.

3 Koren I, Reem E and Kimchi A: Dap1, a novel substrate of mtor, negatively regulates autophagy. Curr Biol 20(12): 1093-1098, 2010. PMID: 20537536 DOI: 10.1016/j.cub.2010.04.041.

4 Chung J, Karkhanis V, Tae S, Yan F, Smith P, Ayers LW, Agostinelli C, Pileri S, Denis GV, Baiocchi RA and Sif S: Protein arginine methyltransferase 5 (prmt5) inhibition induces lymphoma cell death through reactivation of the retinoblastoma tumor suppressor pathway and polycomb repressor complex 2 (prc2) silencing. J Biol Chem 288(49): 35534-35547, 2013. PMID: 24189068 DOI: 10.1074/jbc.M113.510669.

5 Miyazaki T, Shen M, Fujikura D, Tosa N, Kim HR, Kon S, Uede T and Reed JC: Functional role of death-associated protein 3 (dap3) in anoikis. J Biol Chem 279(43): 44667-44672, 2004. PMID: 15302871 DOI: 10.1074/jbc.M408101200.

6 Wybranska I, Polus A, Mikolajczyk M, Knapp A, Sliwa A, Zapala B, Staszel T and Dembinska-Kiec A: Apoptosis-related gene expression in glioblastoma (ln-18) and medulloblastoma (daoy) cell lines. Hum Cell 26(4): 137-148, 2013. PMID: 24037645 DOI: 10.1007/s13577-011-0029-9.

7 Mariani L, Beaudry C, McDonough WS, Hoelzinger DB, Kaczmarek E, Ponce F, Coons SW, Giese A, Seiler RW and Berens ME: Death-associated protein 3 (dap-3) is overexpressed in invasive glioblastoma cells in vivo and in glioma cell lines with induced motility phenotype in vitro. Clin Cancer Res 7(8): 2480-2489, 2001. PMID: 11489830.

8 Wazir U, Sanders AJ, Wazir A, Baig RM, Jiang WG, Ster IC, Sharma AK and Mokbel K: Effect of the knockdown of death-associated protein 1 expression on cell adhesion, growth and migration in breast cancer cells. Oncol Rep 33(3): 1450-1458, 2015. PMID: 25530065 DOI: 10.3892/or.2014.3686.

9 Nie X, Chen H, Niu P, Zhu Y, Zhou J, Jiang L, Li D, Lin M, Chen Z and Shi D: Dap1 negatively regulates autophagy induced by cardamonin in skov3 cells. Cell Biol Int 44(11): 2192-2201, 2020. PMID: 32706448 DOI: 10.1002/cbin.11425.

10 Wazir U, Jiang WG, Sharma AK and Mokbel K: The mrna expression of dap1 in human breast cancer: Correlation with clinicopathological parameters. Cancer Genomics Proteomics 9(4): 199-201, 2012. PMID: 22798505.

11 Jia Y, Ye L, Ji K, Toms AM, Davies ML, Ruge F, Ji J, Hargest R and Jiang WG: Death associated protein 1 is correlated with the clinical outcome of patients with colorectal cancer and has

a role in the regulation of cell death. Oncol Rep 31(1): 175-182, 2014. PMID: 24270644 DOI: 10.3892/or.2013.2866.

12 Jacques C, Fontaine JF, Franc B, Mirebeau-Prunier D, Triau S, Savagner F and Malthiery Y: Death-associated protein 3 is overexpressed in human thyroid oncocytic tumours. Br J Cancer 101(1): 132-138, 2009. PMID: 19536094 DOI: 10.1038/sj.bjc.6605111.

13 Santos M, Maia LL, Silva CV, Peterle GT, Mercante AM, Nunes FD, Carvalho MB, Tajara EH, Louro ID and Silva-Conforti AM: Dap1 high expression increases risk of lymph node metastases in squamous cell carcinoma of the oral cavity. Genet Mol Res 14(3): 10515-10523, 2015. PMID: 26400283 DOI: 10.4238/2015.September.8.13.

14 Sasaki H, Ide N, Yukiue H, Kobayashi Y, Fukai I, Yamakawa Y and Fujii Y: Arg and dap3 expression was correlated with human thymoma stage. Clin Exp Metastasis 21(6): 507-513, 2004. PMID: 15679048 DOI: 10.1007/s10585-004-2153-3.

15 Wazir U, Jiang WG, Sharma AK and Mokbel K: The mrna expression of dap3 in human breast cancer: Correlation with clinicopathological parameters. Anticancer Res 32(2): 671-674, 2012. PMID: 22287761.

16 Jia Y, Ye L, Ji K, Zhang L, Hargest R, Ji J and Jiang WG: Death-associated protein-3, dap-3, correlates with preoperative chemotherapy effectiveness and prognosis of gastric cancer patients following perioperative chemotherapy and radical gastrectomy. Br J Cancer 110(2): 421-429, 2014. PMID: 24300973 DOI: 10.1038/bjc.2013.712.

17 Jia Y, Li Z, Cheng X, Wu X, Pang F, Shi J, Li S, Li X, Hu Y, Zhang L and Ji J: Depletion of death-associated protein-3 induces chemoresistance in gastric cancer cells through the beta-catenin/lgr5/bcl-2 axis. J Investig Med 67(5): 856-861, 2019. PMID: 30792218 DOI: 10.1136/jim-2018-000934.

18 Sato Y, Yoshino H, Kashiwakura I and Tsuruga E: Dap3 is involved in modulation of cellular radiation response by rig-i-like receptor agonist in human lung adenocarcinoma cells. Int J Mol Sci 22(1), 2021. PMID: 33401559 DOI: 10.3390/ijms22010420.

19 Takeda S, Iwai A, Nakashima M, Fujikura D, Chiba S, Li HM, Uehara J, Kawaguchi S, Kaya M, Nagoya S, Wada T, Yuan J, Rayter S, Ashworth A, Reed JC, Yamashita T, Uede T and Miyazaki T: Lkb1 is crucial for trail-mediated apoptosis induction in osteosarcoma. Anticancer Res 27(2): 761-768, 2007.2 PMID: 17465200.

20 Woo Lee J, Hwa Soung Y, Young Kim S, Woo Nam S, Sang Park W, Young Lee J, Jin Yoo N and Hyung Lee S: Mutational analysis of proapoptotic death associated protein 3 (dap3) p-loop domain in common human carcinomas. Acta Oncol 45(4): 489-490, 2006. PMID: 16760188 DOI: 10.1080/02841860500492075.

21 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2021. PMID: 33538338 DOI: 10.3322/caac.21660.

Table I. Pathological information of pancreatic tumours and the expression profiles of DAPs.

| Category | Variables | N= | DAP3 | | DAP1 | |
|---|---|---|---|---|---|---|
| | | | Mean±SD | p value* | Mean±SD | p value* |
| Tissue types | Tumour tissues | 223 | 103.9±22.4 | 0.065 | 725±154 | 0.064 |
| | Normal tissues | 223 | 58±10.6 | | 1574±429 | |
| Gender | Male | 132 | 96.7±27.5 | 0.7 | 872±235 | 0.18 |
| | Female | 91 | 114.9±38.18 | | 502±1478 | |
| Histological types | Adenocarcinoma | 192 | 114.6±25.6 | | 529±121 | |
| | All other types | 29 | 33.98±9.27 | <0.0001 | 2198±886 | 0.15 |
| Differentiation | High | 15 | 51.2±14.2 | | 2764±1644 | |
| | High/Moderate | 20 | 70.7±23.41 | 0.49[a] | 11.63±8.041 | 0.12[a] |
| | Moderate | 77 | 76.8±11.9 | 0.18[a] | 775±220 | 0.26[a] |
| | Moderate/Low | 78 | 138.5±46.94 | 0.079[a] | 380±1854 | 0.18[a] |
| | Low | 15 | 235±211 | 0.4[a] | 966±460 | 0.31[a] |
| Anatomical sites | Head | 73 | 89.4±33.2 | | 1188±376 | |
| | Body | 16 | 71.1±23.2 | 0.65[b] | 227±213 | 0.029[b] |
| | Body and Tail | 32 | 58.8±15.4 | 0.41[b] | 880±370 | 0.56[b] |
| | Tail | 5 | 46.7±14.5 | 0.24[b] | 15.6±15.2 | 0.0027[b] |
| | Other locations | 5 | 52.9±36.5 | 0.48[b] | 1885±1884 | 0.74[b] |
| T staging | T1 | 6 | 55.2±30.55 | | 127±1055 | |
| | T2 | 27 | 69.1±16.1 | 0.7[c] | 169.9±93.4 | 0.77[c] |
| | T3 | 122 | 107±30.2 | 0.25[c] | 833±191 | 0.0021[c] |
| | T4 | 23 | 77±21.32 | 0.59[c] | 426±4142 | 0.49[c] |
| Nodal status | Node Negative | 89 | 153.1±53.41 | | 797±290 | 0.75 |
| | Node Positive | 107 | 72.2±11.3 | 0.14 | 685±195 | |
| TNM staging | TNM-1 | 21 | 64.4±20.6 | | 197±116 | |
| | TNM-2 | 137 | 89.5±21.9[d] | 0.4 | 704±155 | 0.01[d] |
| | TNM-3 | 19 | 74±24.83[d] | 0.64 | 521±506 | 0.54[d] |
| | TNM-4 | 15 | 77.6±23.8[d] | 0.71 | 890±884 | 0.46[d] |
| Microvessel embolism | Without embolism | 126 | 126.4±37.8 | 0.3 | 623±213 | 0.3 |
| | With embolism | 58 | 82.7±18.53 | | 998±2881 | |

[a] *versus* highly differentiated; [b] *versus* tumours of head of pancreas; [c] *versus* T1 tumours; [d] *versus* TNM1 tumours. * by Student t test

Table II. DAP3 expression and nodal status in relation to clinical outcomes

| DAP3 expression | Nodal status | Clinical outcomes | | | |
|---|---|---|---|---|---|
| | | Total | Died of Pancreatic Cancer | Alive | Survival (%) |
| DAP3 low | (-) | 23 | 15 | 8 | 34.8% [a] |
| | (+) | 35 | 17 | 8 | 32.0% [b] |
| DAP3 high | (-) | 42 | 31 | 11 | 26.2% [c] |
| | (+) | 69 | 65 | 4 | 5.8% |

[a]p=0.0013, [b]p=0.0022, [c]p=0.0036 *versus* DAP3 high/node(+), by Fisher exact test

Table III. Clinical, pathological and DAP3 expression in predicting the mortality of patients*.

| **Factors** | **P value*** | **HR** |
|---|---|---|
| Gender | 0.664 | 0.734 |
| Age | 0.293 | 1.035 |
| Histological Type | 0.598 | 0.537 |
| Differentiation | 0.825 | 0.918 |
| Location of tumours | 0.92 | 1.042 |
| Local Invasion | 0.631 | 1.846 |
| Nodal status | 0.18 | 0.256 |
| TNM staging | 0.769 | 0.624 |
| Tumour vascular embolism | 0.644 | 0.657 |
| DAP3 | 0.296 | 0.372 |
| DAP3/Nodal status | 0.006 | 10.036 |

* Multivariate analysis against death of the patients.

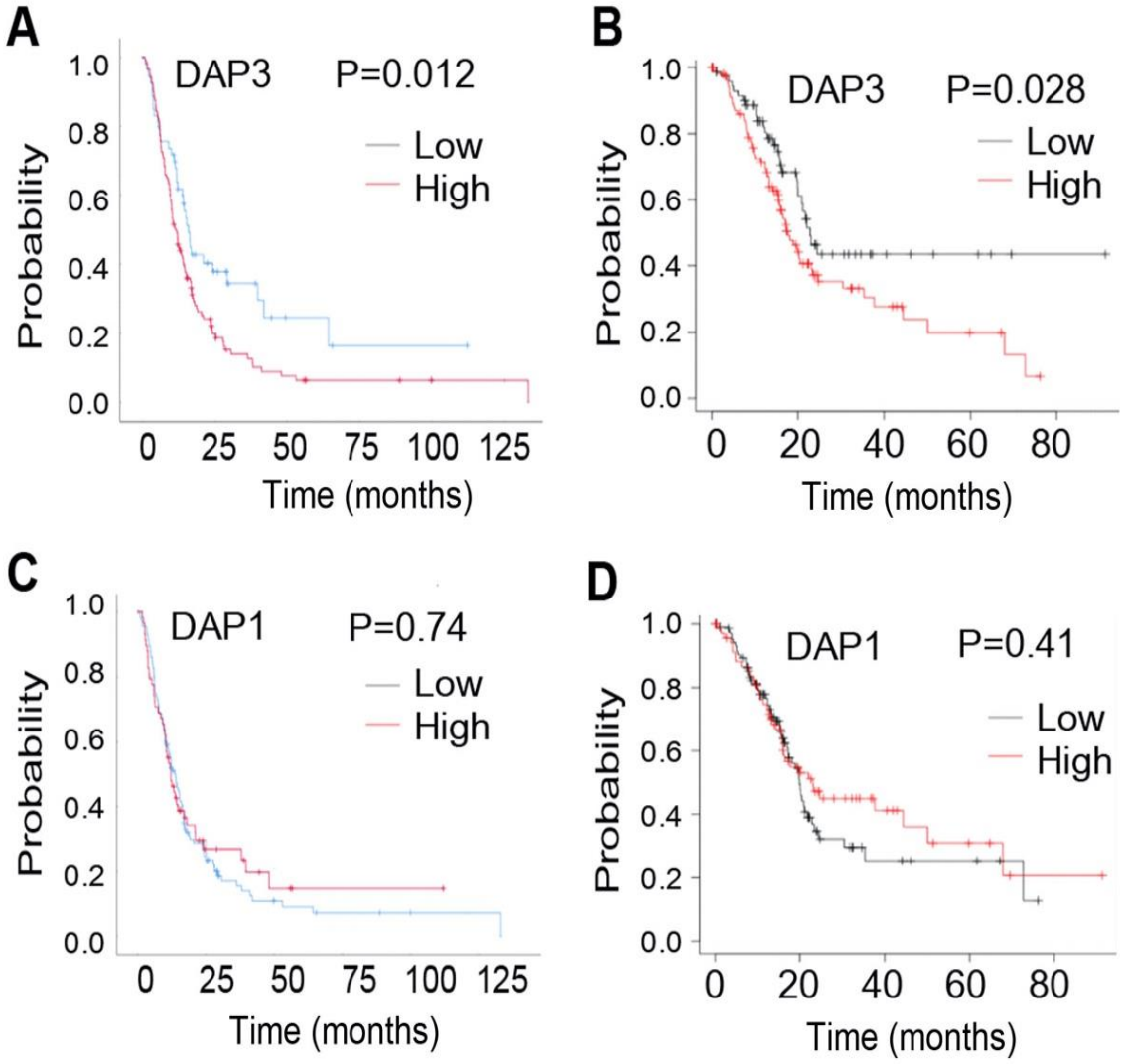


Figure 1. DAP3 and DAP1 expression and the overall survival of the patients in the host cohort (A, DAP3; C, DAP1)) and TCGA cohort (B, DAP3; D, DAP1). Kaplan Meier survival analysis. P value as indicated in the figure.

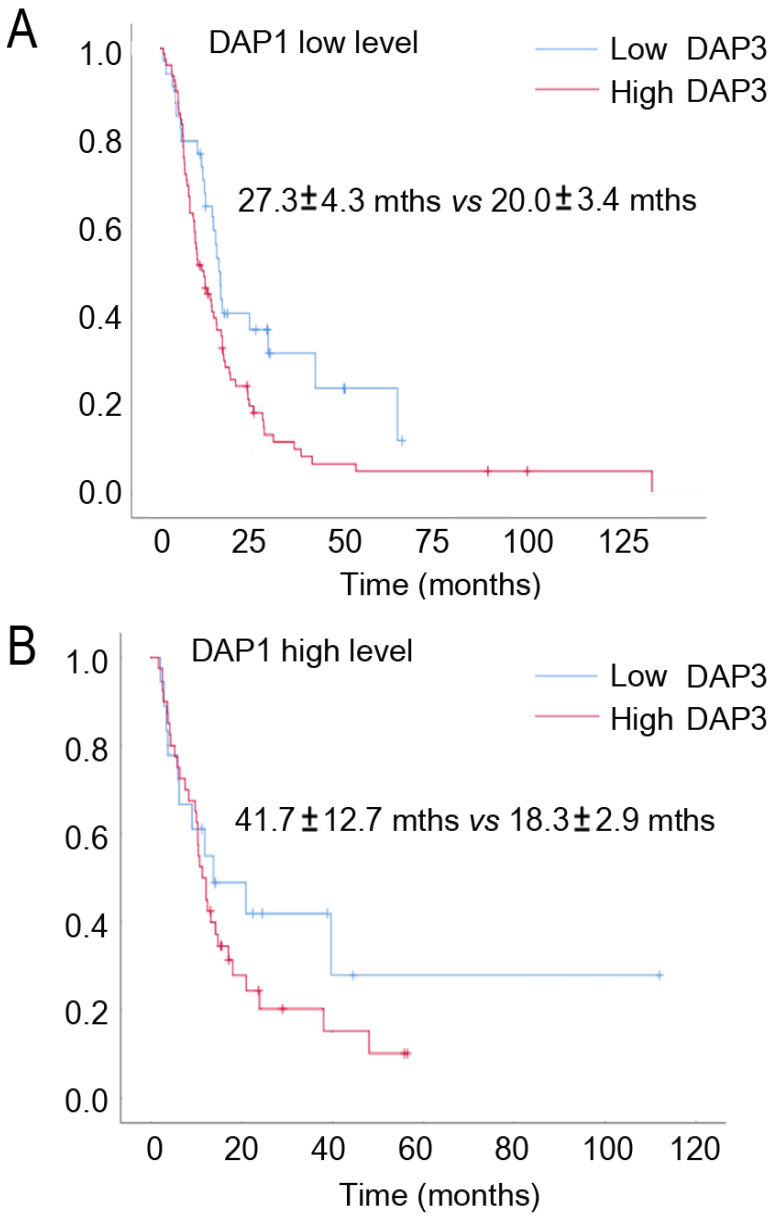


Figure 2. Overall survival and DAP3 expression as stratified by DAP1 (A, DAP1 low levels; B, DAP1 high levels). The grouping has showed a significant relationship between DAP3 and survival in both groups by Kaplan-Meier survival analysis ($p$=0.024).

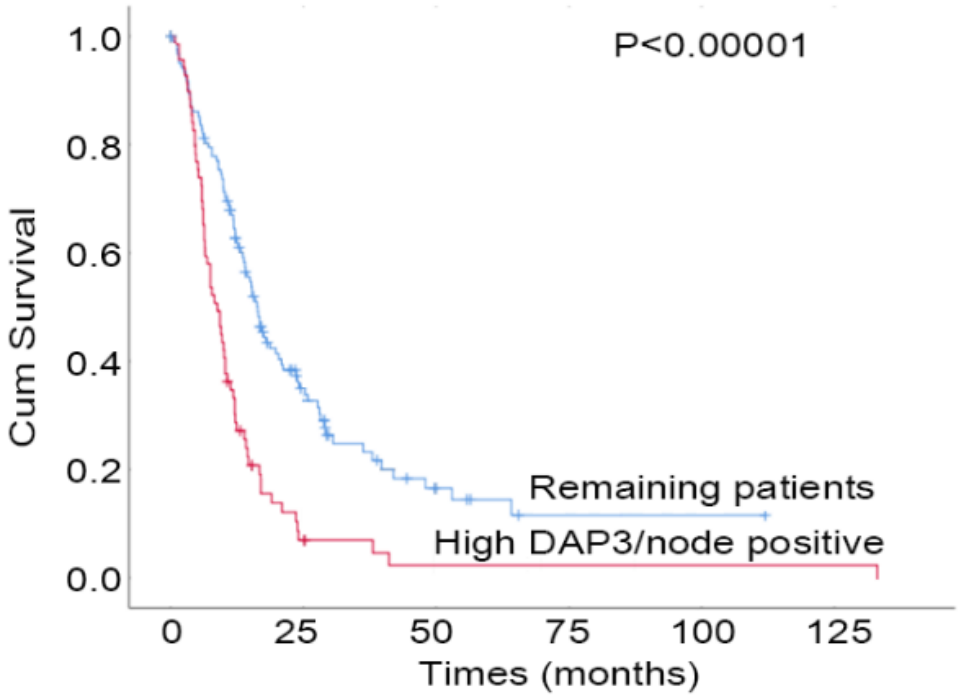


Figure 3. DAP3 expression and nodal status strengthened the predictive power of overall survival of the patients by Kaplan-Meier survival analysis ($p<0.00001$).