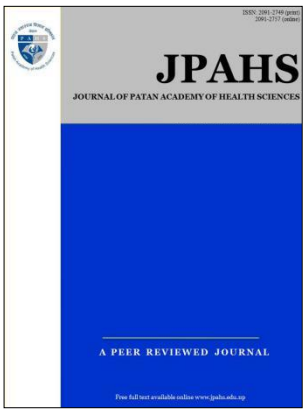

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Membrane expression and significance of TRAIL death receptors DR4 and DR5 in Pancreatic cancer

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Abstract

Introduction: Tumor necrosis factor related-apoptosis-inducing ligand (TRAIL) is a powerful and selective activator of apoptosis in many cancer cells. We aim to investigate the expression and significance of TRAIL death receptor DR4 and DR5 in pancreatic cancer (PC) tissues.

Method: Twenty-eight histologically verified samples of PC tissue were collected between 2018 and 2019. TRAIL death receptor expression profiles were determined by immunohistochemistry.

Result: Death receptor DR4 and DR5 were expressed in the PC tissue and the adjacent non-cancerous pancreatic tissues, the expression of DR4 and DR5 in the PC tissue was significantly higher than that of the adjacent non-cancerous pancreatic tissues ($p<0.05$). Additionally, in both the tissue group, the expression of DR4 was significantly stronger than the DR5 ($p<0.05$). To assess the relationship between DR4 and DR5 expression, differentiation, and tumor staging of PC, the result reveals that the expression of DR4 and DR5 was significantly higher in stage I tumors than the stage II, III, IV tumors ($p<0.05$). In contrast, the expression of DR4 and DR5 was decreased with a decrease in the degree of differentiation of tumors. However, the difference was not statistically significant.

Conclusion: The membrane expression of TRAIL death receptor DR4 and DR5 is greater in PC than in the adjacent non-cancerous pancreatic tissues. Furthermore, increased membrane expression of TRAIL death receptor DR4 and DR5 in stage I PC and well-differentiated PC may predict the prognosis and feasibility of using TRAIL gene therapy as a treatment option for early PC.

Keywords: apoptosis, death receptors, pancreas cancer, TRAIL

Introduction

Pancreatic cancer (PC) is deadly cancer and the fourth leading cause of cancer death in the United States.[1] Even with the multidisciplinary treatment approach the outcome of patients with PC has been unsatisfactory with a five-year survival of <5%.[2-9]

The advancement of the tumor is associated with the dysfunction of apoptosis.[10-13] Recently, tumor necrosis factor related-apoptosis-inducing ligand (TRAIL), a type II membrane protein, is a powerful and selective activator of apoptosis in many cancer cells.[14-18] It interacts with five different death receptors: TRAIL-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 (DcR1), TRAIL-R4 (DcR2), and osteoprotegerin. Death receptors DR4 and DR5 are membrane-bound receptors and contains the death domain in its intracellular portion that signals for apoptosis. In contrast, DcR1, DcR2, and osteoprotegerin are soluble receptors and do not contain a death domain; thus, they are unable to transmit the apoptotic signal.[19-21] The expression of TRAIL and its receptors has widely been studied in normal and cancerous tissues.[22-27] Moreover, studies have also revealed that loss of TRAIL receptor expression correspond with bad prognosis and tumor recurrence.[27-33]

We aim to assess the membrane expression of DR4 and DR5 in PC tissues and adjacent non-cancerous pancreatic tissue by immunohistochemistry (IHC).

Method

Tissue samples were obtained from patients already diagnosed with PC, who underwent pancreatic resection between 2018 to 2019 at Lihuili Hospital, Ningbo, China. Clinical and pathological characteristics were obtained from the medical records. Original pathology reports, including age, histological tumor type and grade, tumor size, and lymph node status were analyzed. Freshly removed tissue samples were immediately fixed in paraformaldehyde solution for 12-24 hours and paraffin-embedded for IHC. The study was approved by the Human Subject Committee of the Lihuili Hospital and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Consent was obtained from all individual participants included in the study.

Immunohistochemistry (IHC): Tissue sections (5 µm thickness) were prepared, deparaffinized in xylene, and hydrated using an ethanol gradient. Antigen retrieval and IHC were performed. Antigen retrieval was performed for both DR4 and DR5 by microwave treatment of the slides at 1000 W in 1 L distilled water with phosphate-buffered saline (PBS) and heated to a boiling point, the power was cut off and the process was repeated after 10 minutes of interval, the slide was then rinsed with PBS twice after cooling. Endogenous peroxidase was blocked with 0.3% H_2O_2 in PBS solution [6.4 mM $Na_2HPO_4H_2O$, 1.5 mM KH_2PO_4, 0.14 M NaCl, and 2.7 mM KCl (pH 7.8)] for 10 min at room temperature, and was rinsed twice with PBS, followed by incubation with primary antibody (DR4 and DR5 antibody) diluted with PBS (1:100) for 1 hour at 37°C or 4°C overnight and was rinsed in PBS (3×2 min). After washing with PBS, the slides were incubated with a 1:100 dilution of a biotinylated rabbit-antigoat antibody (DAKO) for 25 min and was rinsed in PBS (3×2 min), followed by the addition of a drop of A and B reagent (DAKO) into 1mL distilled water respectively , the solution was mixed and dropped to the sections. The microscope was used to observe the DAB chromogenic reaction, the chromogenic reaction was controlled on time by rinsing the slide with distilled water. Counterstaining was performed with hematoxylin for 2 min. The slide was further rinsed with distilled water, dried completely with a drier and mounted with natural gum, and covered with a slit. Thus, the slide was ready for microscopic examination.

Immunohistochemical scoring of DR4 and DR5: Tissue sections were analyzed by a single pathologist with no prior knowledge of the

patient status or antibodies used. The calculation of the final immunohistochemical staining scores in pancreatic tissues included both intensity and marker distribution (percentage of the positively stained epithelial cells). The intensity of the pancreatic tissue staining was assessed as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. Moreover, marker distribution was calculated as 0, less than 10%; 1, 10% to 40%; 2, 40% to 70%; and 3, more than 70% of the epithelial cells stained on the sections. Summing the scores of both the intensity and the marker distribution for a given patient resulted in the final immunostaining score.

Statistical analyses were performed using SPSS 18.0 statistical data processing software, the data were analysed by Non-parametric tests and the SEM is displayed as error bars for all data points in all of the figures.

Result

A total of 28 PC tissue samples were obtained from patients already diagnosed with PC. The median age of the patients was 63 years. There were 5 cases of well-differentiated, 12 cases of medium differentiated, and 11 cases of poorly differentiated PC in which 7 cases were in stage I (cancer confined to the pancreas), and the rest 21 cases in stage II, stage III and stage IV in combined (cancer invaded to surrounding tissue other than the pancreas).

Expression of DR4 and DR5 in the pancreatic cancer tissue and the adjacent non-cancerous pancreatic tissues: DR4 and DR5 were expressed in the PC tissue and the adjacent non-cancerous pancreatic tissues, Figure 1A, B, C, D. The expression of DR4 and DR5 in the PC tissue was significantly higher than that of the adjacent non-cancerous pancreatic tissues ($p < 0.05$), Figure 2. In both the tissue group, the expression of DR4 was significantly higher than the DR5 ($P < 0.05$).

Relationship between DR4 and DR5 expression, differentiation, and staging of PC: The results for the relationship between DR4 and DR5 expression, differentiation and tumor staging of PC shows that the expression of DR4 and DR5 was significantly higher in non-lymph node metastasis (stage I) tumors than the lymph node metastasis (stage II, III, IV) tumors ($p<0.05$), Figure 3. In contrast, expression of DR4 and DR5 was decreased with a decrease in degree of differentiation of tumors, Figure 4, but the difference was not statistically significant.

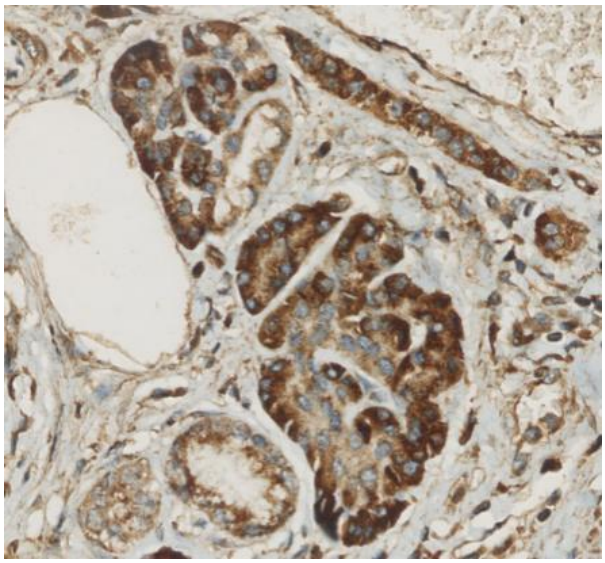

Figure 1A

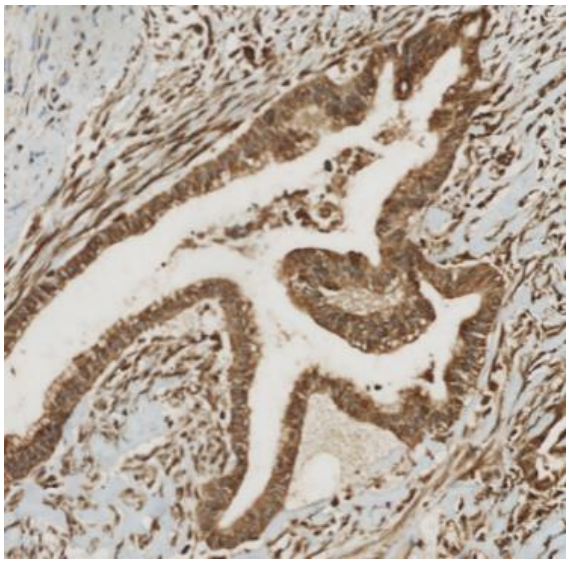

Figure 1B

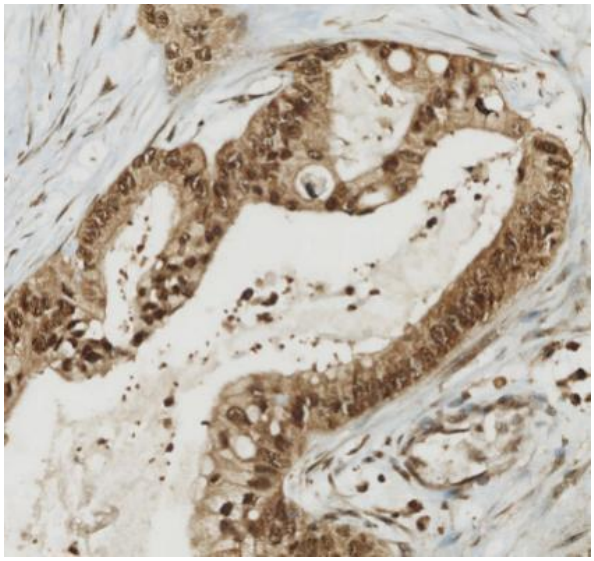

Figure 1C

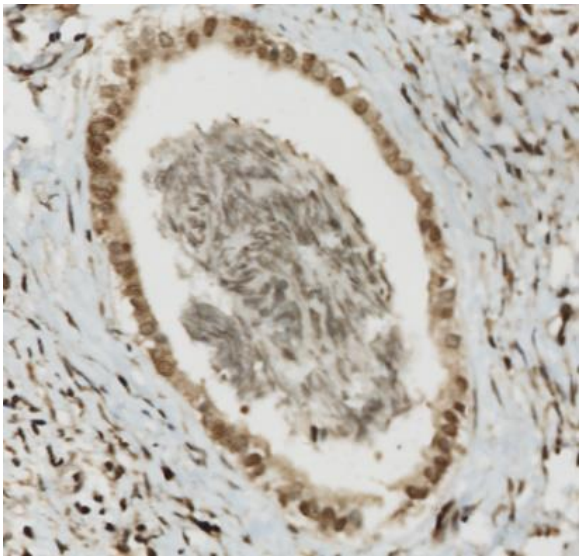

Figure 1D

Figure 1A-D . Expression of DR4 and DR5 in PC tissue and adjacent non-cancerous tissue

A. Positive membrane expression of DR4 in PC; B. Positive membrane expression of DR4 in adjacent non-cancerous pancreatic tissues; C. Positive membrane expression of DR5 in PC; D. Positive membrane expression of DR4 in adjacent non-cancerous pancreatic tissues.

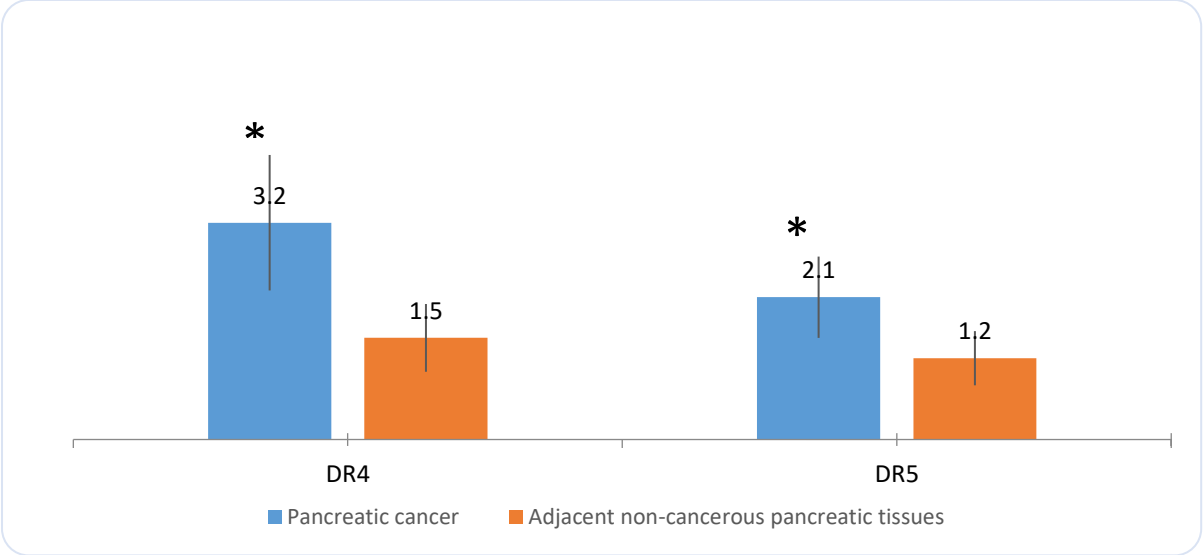

Figure 2. Qualitative analysis of immunohistochemical expression of DR4 and DR5 in PC tissue versus adjacent non-cancerous pancreatic tissue.

Note: Immunohistochemical scoring (mean±SEM) was performed as described in the materials and methods using the indicated antibodies. Asterisk indicates statistically significant differences among both the groups.

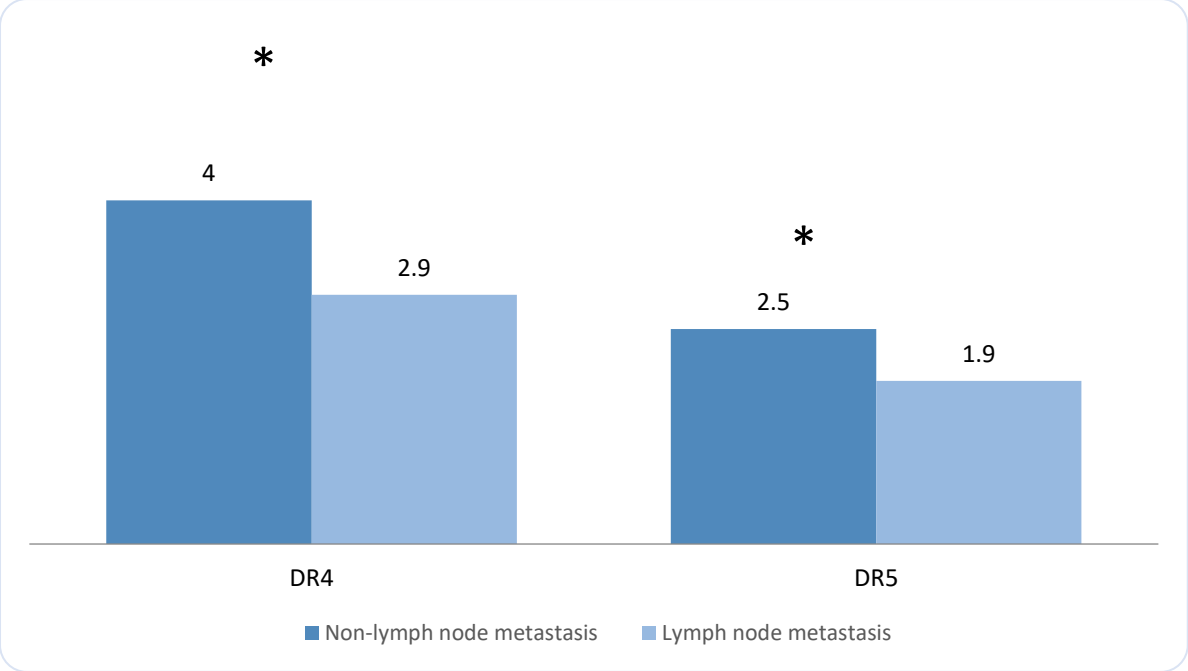

Figure 3. Qualitative analysis of immunohistochemical expression (mean±SEM) of DR4 and DR5 in non-lymph node metastasis (Stage I) and lymph node metastasis (Stage II, III, and IV) PC.
*Asterisk indicates statistically significant differences among both the groups.

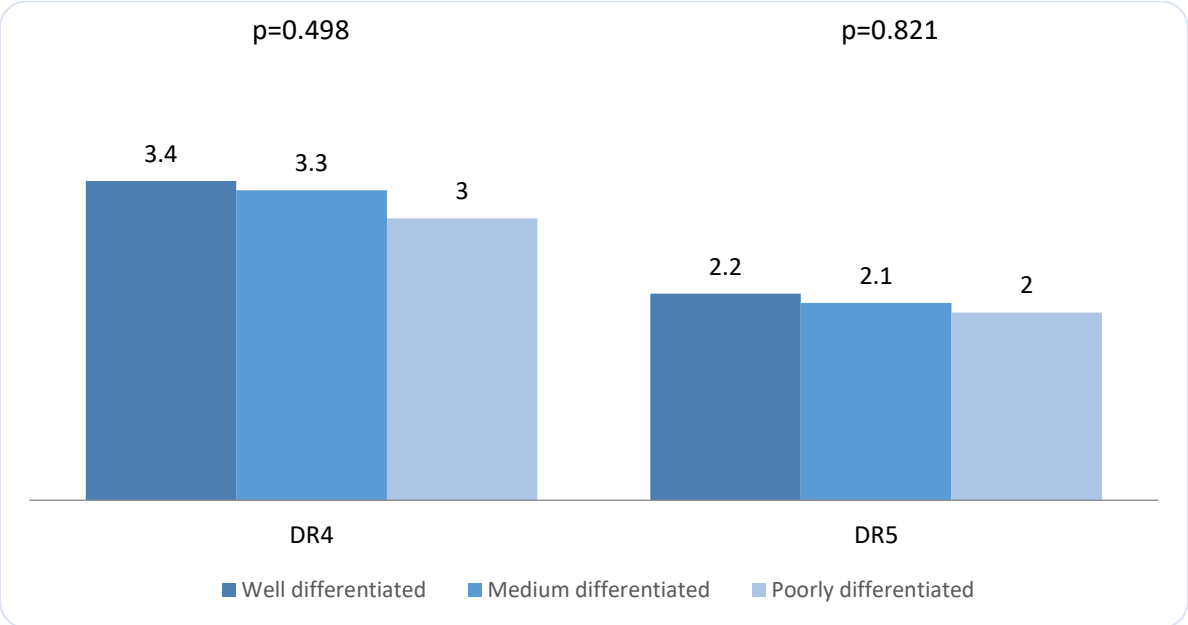

Figure 4. Qualitative analysis of immunohistochemical expression (mean±SEM) of DR4 and DR5 in well-differentiated, medium differentiated, and poorly differentiated PC.
Note: The finding was not statistically significant between both the groups.

Discussion

Pancreatic cancer is one of the most devastating malignant cancer and the fourth leading cause of cancer death.[1] Very little progress has been achieved in the treatment of PC in the last 25 years, possibly because PC harboring a complex network of mutated genes and also has a strong ability to resist apoptosis.[2,34]

TRAIL as a powerful and selective activator of apoptosis in many cancer cells with minimal effect on normal cells and as a potent cancer preventive negotiator has attracted researchers to use the TRAIL gene as an anti-cancer therapy in the clinical practice.[14-18] The TRIAL binds to DR4 and DR5 receptors and initiates the formation of a protein complex called the death-inducing signaling complex (DISC), which further is responsible to induce apoptosis through a chain of steps.[35] However, decoy receptors present on the cancer cells can inhibit the apoptosis induced by TRAIL.[34] In past years, many studies have successfully reported potential clinical use of rhTRAIL in different cancers.[29-32] Nonetheless, many areas of TRAIL as an anti-cancer therapy is still yet to be explored.

Our study confirmed that TRAIL death receptors DR4 and DR5 are expressed in both the PC tissue and the adjacent non-cancerous pancreatic tissues. Encouragingly, several previous studies have reported the expression of DR4 and DR5 in the cell membrane, cytoplasm, nucleus, normal and cancerous cells.[22-27,36] Besides, our study also found that the expression of DR4 and DR5 was significantly higher in stage I tumors than that of stage II, III, or IV tumors. This finding was consistent with previous studies, where it has revealed that the loss of DR4 and DR5 expression in cancerous tissue leads to poor prognosis, recurrence, and progression of cancer.[28,37] Furthermore, the loss in expression of TRAIL death receptor (DR4 and DR5) in late stages of PC has been associated with TRAIL resistance.[33,38] A recent study revealed that some PC cells use DR4 to induce cell death, whereas other PC cells such as AsPC-1 and BxPC-3 cells trigger apoptosis through DR5.[39] Another research demonstrated that drozitumab, a human agonistic monoclonal antibody binds with DR5 and selectively eliminates cancer stem cells in patient-derived pancreatic tumor xenografts (PDX) model, resulting in regression of PC and long-term tumor control.[40] Thus, the TRAIL death receptor expression in PC is an important target for the success of PC treatment.

The limitation of this study is the small sample size and demands further prospective studies in larger populations to confirm these results and to assess its value in clinical practice.

Conclusion

The membrane expression of TRAIL death receptor DR4 and DR5 was greater in PC than the adjacent non-cancerous pancreatic tissues. The increased membrane expression of TRAIL death receptor DR4 and DR5 in stage I PC and well-differentiated PC may predict the prognosis and feasibility of using TRAIL gene therapy as a treatment option for early PC. The increased membrane expression of TRAIL death receptor DR4 and DR5 in stage I PC and well-differentiated PC may predict the prognosis and feasibility of using TRAIL gene therapy as a treatment option for early PC.

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Conflict of Interest

The authors declare that they have no competing interests.

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Author Contribution

Study design: DKY and CDL; Data collection: DKY, ZSW, and YFH; Data analysis: DKY and ZSW; Preparation of the manuscript: DKY and ZSW; Final draft review: All authors

Reference

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA: a cancer journal for clinicians. 2017;67(1):7-30. | DOI | PubMed | Google Scholar |
2. Yadav DK, Bai X, Yadav RK, Singh A, Li G, Ma T, et al. Liquid biopsy in pancreatic cancer: the beginning of a new era. Oncotarget.

2018;9(42):26900-33. | DOI | PubMed | Google Scholar | Full Text | Weblink |
3. Yadav DK LC, Yadav RK. Vaccine Therapy for Pancreatic Cancer: A Battle against Deadly Cancer. J Cancer Sci Ther2014. p. 268-77 . | DOI | Google Scholar | Full Text | Weblink |
4. Rahib L, Fleshman JM, Matrisian LM, Berlin JD. Evaluation of Pancreatic Cancer Clinical Trials and Benchmarks for Clinically Meaningful Future Trials: A Systematic Review. JAMA oncology. 2016;2(9):1209-16. | DOI | PubMed | Google Scholar | Weblink |
5. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. The New England journal of medicine. 2011;364(19):1817-25. | DOI | PubMed | Google Scholar | Weblink |
6. da Rocha Lino A, Abrahão CM, Brandão RM, Gomes JR, Ferrian AM, Machado MCC, et al. Role of gemcitabine as second-line therapy after progression on FOLFIRINOX in advanced pancreatic cancer: a retrospective analysis. Journal of Gastrointestinal Oncology. 2015;6(5):511-5. | DOI | PubMed | Google Scholar | Full Text | Weblink |
7. Wang Y, Hu G, Zhang Q, Tang N, Guo J, Liu L, et al. Efficacy and safety of gemcitabine plus erlotinib for locally advanced or metastatic pancreatic cancer: a systematic review and meta-analysis. Drug Design, Development and Therapy. 2016;10:1961-72. | DOI | PubMed | Google Scholar | Weblink |
8. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature. 2016;531(7592):47-52. | DOI | PubMed | Weblink |
9. Conlon KC, Klimstra DS, Brennan MF. Long-term survival after curative resection for pancreatic ductal adenocarcinoma. Clinicopathologic analysis of 5-year survivors. Annals of surgery. 1996;223(3):273-9. | DOI | PubMed | Weblink |
10. Srinivasan S, Guha M, Kashina A, Avadhani NG. Mitochondrial dysfunction and mitochondrial dynamics-The cancer connection. Biochimica et biophysica acta. 2017;1858(8):602-14. | DOI | PubMed | Weblink |
11. Trotta AP, Chipuk JE. Mitochondrial dynamics as regulators of cancer biology. Cellular and molecular life sciences : CMLS. 2017;74(11):1999-2017. | DOI | PubMed | Weblink |
12. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity. 1995;3(6):673-82. | DOI | PubMed | Full Text |
13. Wajant H, Pfizenmaier K, Scheurich P. TNF-related apoptosis inducing ligand (TRAIL) and its receptors in tumor surveillance and cancer therapy. Apoptosis : an international journal on programmed cell death. 2002;7(5):449-59. | DOI | PubMed | Weblink |
14. Zhang L, Ren X, Alt E, Bai X, Huang S, Xu Z, et al. Chemoprevention of colorectal cancer by targeting APC-deficient cells for apoptosis. Nature. 2010;464(7291):1058-61. | DOI | PubMed | Weblink |
15. Johnstone RW, Frew AJ, Smyth MJ. The TRAIL apoptotic pathway in cancer onset, progression and therapy. Nature reviews Cancer. 2008;8(10):782-98. | DOI | PubMed | Weblink|
16. Borja-Cacho D, Yokoyama Y, Chugh RK, Mujumdar NR, Dudeja V, Clawson KA, et al. TRAIL and Triptolide: An Effective Combination that Induces Apoptosis in Pancreatic Cancer Cells. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2010;14(2):252-60. | DOI | PubMed |Weblink |
17. Mahalingam D, Natoni A, Keane M, Samali A, Szegezdi E. Early growth response-1 is a regulator of DR5-induced apoptosis in colon cancer cells. British journal of cancer. 2010;102(4):754-64.| DOI | PubMed| Weblink|
18. Szliszka E, Czuba ZP, Kawczyk-Krupka A, Sieron-Stoltny K, Sieron A, Krol W. Chlorin-based photodynamic therapy enhances the effect of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in bladder cancer cells. Medical science monitor : international medical journal of experimental and clinical research. 2012;18(1):Br47-53. | DOI | PubMed | Weblink|
19. Newsom-Davis T, Prieske S, Walczak H. Is TRAIL the holy grail of cancer therapy? Apoptosis : an international journal on programmed cell death. 2009;14(4):607-23. | DOI | Weblink |
20. Griffith TS, Rauch CT, Smolak PJ, Waugh JY, Boiani N, Lynch DH, et al. Functional analysis of TRAIL receptors using monoclonal antibodies. Journal of immunology (Baltimore, Md : 1950). 1999;162(5):2597-605. | PubMed | Full Text | Weblink |
21. Wu GS. TRAIL as a target in anti-cancer therapy. Cancer letters. 2009;285(1):1-5. | DOI | PubMed | Weblink |
22. Frank S, Kohler U, Schackert G, Schackert HK. Expression of TRAIL and its receptors in human brain tumors. Biochemical and biophysical research communications. 1999;257(2):454-9. | DOI | PubMed | Weblink |

23. Spierings DC, de Vries EG, Vellenga E, van den Heuvel FA, Koornstra JJ, Wesseling J, et al. Tissue distribution of the death ligand TRAIL and its receptors. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 2004;52(6):821-31. | DOI | PubMed | Weblink |
24. Koornstra JJ, Kleibeuker JH, van Geelen CM, Rijcken FE, Hollema H, de Vries EG, et al. Expression of TRAIL (TNF-related apoptosis-inducing ligand) and its receptors in normal colonic mucosa, adenomas, and carcinomas. The Journal of pathology. 2003;200(3):327-35. | DOI | PubMed | Weblink |
25. Younes M, Georgakis GV, Rahmani M, Beer D, Younes A. Functional expression of TRAIL receptors TRAIL-R1 and TRAIL-R2 in esophageal adenocarcinoma. European journal of cancer (Oxford, England : 1990). 2006;42(4):542-7. | DOI | PubMed |Weblink |
26. Ganten TM, Sykora J, Koschny R, Batke E, Aulmann S, Mansmann U, et al. Prognostic significance of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor expression in patients with breast cancer. Journal of molecular medicine (Berlin, Germany). 2009;87(10):995-1007. | DOI | PubMed | Weblink |
27. Yoldas B, Ozer C, Ozen O, Canpolat T, Dogan I, Griffith TS, et al. Clinical significance of TRAIL and TRAIL receptors in patients with head and neck cancer. Head & neck. 2011;33(9):1278-84. | DOI | PubMed |Weblink |
28. Kriegl L, Jung A, Engel J, Jackstadt R, Gerbes AL, Gallmeier E, et al. Expression, cellular distribution, and prognostic relevance of TRAIL receptors in hepatocellular carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2010;16(22):5529-38. | DOI | PubMed | Weblink |
29. Strebel A, Harr T, Bachmann F, Wernli M, Erb P. Green fluorescent protein as a novel tool to measure apoptosis and necrosis. Cytometry. 2001;43(2):126-33. | DOI | PubMed |Weblink |
30. Kim JY, Kim YM, Park JM, Han YM, Lee KC, Hahm KB, et al. Cancer preventive effect of recombinant TRAIL by ablation of oncogenic inflammation in colitis-associated cancer rather than anticancer effect. Oncotarget. 2018;9(2):1705-16. | DOI | PubMed | Weblink|
31. Bellail AC, Qi L, Mulligan P, Chhabra V, Hao C. TRAIL agonists on clinical trials for cancer therapy: the promises and the challenges. Reviews on recent clinical trials. 2009;4(1):34-41. | DOI | PubMed | Weblink |
32. Stuckey DW, Shah K. TRAIL on Trial: Preclinical advances for cancer therapy. Trends in molecular medicine. 2013;19(11). | DOI | PubMed | Weblink |
33. Katz MH, Spivack DE, Takimoto S, Fang B, Burton DW, Moossa AR, et al. Gene therapy of pancreatic cancer with green fluorescent protein and tumor necrosis factor-related apoptosis-inducing ligand fusion gene expression driven by a human telomerase reverse transcriptase promoter. Annals of surgical oncology. 2003;10(7):762-72. | DOI | PubMed | Weblink |
34. Wang W, Zhang M, Sun W, Yang S, Su Y, Zhang H, et al. Reduction of decoy receptor 3 enhances TRAIL-mediated apoptosis in pancreatic cancer. PloS one. 2013;8(10):e74272. | DOI | PubMed | Weblink|
35. Bratton SB, MacFarlane M, Cain K, Cohen GM. Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. Experimental cell research. 2000;256(1):27-33. | DOI | PubMed | Weblink|
36. Gottwald L, Piekarski J, Kubiak R, Szwalski J, Pasz-Walczak G, Sęk P, et al. Membrane expression of TRAIL receptors DR4, DR5, DcR1 and DcR2 in the normal endometrium, atypical endometrial hyperplasia and endometrioid adenocarcinoma: a tissue microarray study. Archives of Gynecology and Obstetrics. 2013;288(4):889-99. | DOI |PubMed |Weblink|
37. Li Y, Jin X, Li J, Jin X, Yu J, Sun X, et al. Expression of TRAIL, DR4, and DR5 in bladder cancer: correlation with response to adjuvant therapy and implications of prognosis. Urology. 2012;79(4):968.e7-15. | DOI | PubMed | Weblink |
38. Khanbolooki S, Nawrocki ST, Arumugam T, Andtbacka R, Pino MS, Kurzrock R, et al. Nuclear factor-kappaB maintains TRAIL resistance in human pancreatic cancer cells. Molecular cancer therapeutics. 2006;5(9):2251-60. | DOI | PubMed |Weblink |
39. Mohr A, Yu R, Zwacka RM. TRAIL-receptor preferences in pancreatic cancer cells revisited: Both TRAIL-R1 and TRAIL-R2 have a licence to kill. BMC cancer. 2015;15:494. | DOI | PubMed | Weblink |
40. Eng JW, Mace TA, Sharma R, Twum DYF, Peng P, Gibbs JF, et al. Pancreatic cancer stem cells in patient pancreatic xenografts are sensitive to drozitumab, an agonistic antibody against DR5. Journal for immunotherapy of cancer. 2016;4:33. | DOI | PubMed |Weblink |