Inhibition of the growth of human melanoma cells by methionine enkephalin

DONG-MEI WANG¹, GUANG-CHUAN WANG², JING YANG³, NICOLAS P. PLOTNIKOFF⁴, NOREEN GRIFFIN⁵, YU-MAN HAN¹, RUI-QUN QI⁵, XING-HUA GAO⁵ and FENG-PING SHAN¹

¹Department of Immunology, School of Basic Medical Science, China Medical University, Shenyang, Liaoning 110122; ²Departments of Immunology and ³Pathology, School of Basic Medical Science, Liaoning Medical University, Jinzhou, Liaoning 121000, P.R. China; ⁴Immune Therapeutics, Inc., Orlando, FL 32801, USA; ⁵Department of Dermatology, No. 1 Hospital, China Medical University, Shenyang, Liaoning 110001, P.R. China

Received October 16, 2015; Accepted August 30, 2016

DOI: 10.3892/mmr.2016.5941

Abstract. Melanoma is an aggressive cancer, the incidence of which is increasing worldwide. Limited therapies are currently available, particularly following metastasis. The aim of the present study was to investigate the inhibiting effect of methionine enkephalin (MENK) on human melanoma via opioid receptors. The results of the present study revealed that MENK markedly regulates the proliferation of A375 cells, causing cell cycle arrest in G0/G1 phase and a decrease in the percentage of cells in S and G2/M phases. Reverse transcription-quantitative polymerase chain reaction demonstrated that MENK treatment increased opioid receptor expression in A375 cells. Furthermore, the expression level of survivin, an inhibitory apoptotic protein, was 1.1% of the level in the control group in the MENK group following 48 h of treatment. In conclusion, the results of the present study revealed, to the best of our knowledge for the first time, that MENK may inhibit growth and induce apoptosis of A375 cells, and describes a potential mechanism underlying these effects. Therefore, MENK should be investigated as a primary therapy for human melanoma cancer and as an adjuvant to other chemotherapies. Further studies are required to develop an optimal strategy for the use of MENK for the treatment of human cancers.

Introduction

Human melanoma is the most malignant skin cancer (1) and its incidence has risen rapidly in recent years. It is the most common skin cancer in females aged 25-29 (2). The median survival for patients with metastatic melanoma is ~7 months (3,4); surgery is currently the primary treatment. Drug resistance is a common problem associated with anticancer drugs (5,6). Natural products and medicines have been developed as cosmeceutical ingredients to treat esthetic skin problems, including skin darkening and wrinkles (7-10). Therefore, it may be beneficial to develop natural therapeutic agents for the treatment of melanoma.

Methionine enkephalin (MENK), an endogenous opioid neuropentapeptide composed of Tyr-Ala-Ala-Phe-Met, is derived from pre-enkephalin and circulates in the blood at low concentrations. Various types of opioid receptors have been described and two of the most studied are µ (MOR) and δ (DOR) (11,12). These receptors are expressed on the surface of immune cells and various tumor cells, including pancreatic cancer and melanoma cells (13,14). MENK upregulates the activity of immune cells and may inhibit tumor growth (15,16). MENK and opioid receptors form a biological axis that regulates cell proliferation by delaying G1/S cell cycle progression under homeostatic conditions and in neoplasia, and inhibits pancreatic tumor progression (17). However, the molecular mechanism underlying this effect remains unclear. The present study aimed to investigate the mechanism underlying the therapeutic effect of MENK on melanoma, to determine whether MENK may be a potential therapeutic strategy for the treatment of cancer and other conditions in which the immune system is suppressed.

Materials and methods

Cell culture. A375 human melanoma cells were provided by No. 1 Hospital, China Medical University (Shenyang, China), and cultured in RPMI 1640 (Gibco; Thermo Fisher Scientific,
Inc., Waltham, MA, USA), supplemented with 10% fetal calf serum (FCS; Biological Industries, Kibbutz Beit Haemek, Israel), 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were cultured at 37°C and 5% CO2, and in the exponential phase of growth were used for subsequent experiments.

Reagents. MENK (≥97% purity) was provided by Penta Biotech, Inc. (Union City, CA, USA). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) kits were purchased from Takara Bio, Inc. (Otsu, Japan). TRIzol® was obtained from Invitrogen; Thermo Fisher Scientific, Inc. Propidium iodide (PI), dimethylsulfoxide and thymidine were purchased from Sigma-Aldrich; Merck Millipore (Darmstadt, Germany).

Cell culture and MENK administration in vitro. A375 cells were treated with various concentrations of MENK (0, 2.5, 5, 10 and 12.5 mg/ml in RPMI 1640) for various times (24, 48, 72 and 96 h). Cell viability was evaluated by MTS assays and flow cytometry.

Morphologic observation of A375 cells. Cells were treated with 12.5 mg/ml MENK for 48 h and alterations in morphology were observed under a conventional light microscope.

Cell growth and cell cycle analysis. A375 cells were collected as target cells and seeded into 96-well plates (3x104/well). The A375 proliferation in each well was determined 3 h following plating by measuring the optical density at 570 nm using a bichromatic microplate reader.

A375 cells were seeded in 6-well plates for 48 h (5x104/well). The cells were trypsinized, washed with phosphate-buffered saline (PBS) and fixed in 70% ethanol. Prior to flow cytometric analysis, fixed cells were stained with 0.5 mg/ml PI in PBS containing 50 µg/ml RNase A. Cells were acquired on a flow cytometer and the data was analyzed using ModFit LT™ software version 4.0 (BD Biosciences, Franklin Lakes, NJ, USA).

RT-qPCR analysis of MENK-associated opioid receptors. The mRNA expression levels of MOR, DOR and κ-opioid receptors (KOR) were detected by RT-qPCR. A375 cells (3x104/well) were divided into 3 groups: MENK (12.5 mg/ml), MENK with naltrexone (NTX; 1 mg/ml; Penta Biotech, Inc., Union City, CA, USA) and control. Cells were cultured for 48 h. Total RNA was extracted from cells of the 3 groups using TRIzol, and cDNA was synthesized using reverse transcriptase. Aliquots of cDNA were used as the template for qPCR reactions containing primers for MOR, DOR, KOR or β-actin. The primers were synthesized by Invitrogen; Thermo Fisher Scientific, Inc. and had the following oligo-nucleotide sequences: Forward, 5'-TGTTTACGG-3' for β-actin. The qPCR reactions were performed using an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc.) and SYBR® Premix EX Taq II. The cycling conditions were as follows: An initial denaturation step at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 15 sec and annealing at 60°C for 1 min. Data were normalized to β-actin using the 2ΔΔCq method (18).

Apoptosis analysis. Apoptosis was assessed by labeling A375 cells with Annexin V-fluorescein isothiocyanate (FITC) and PI (BD Biosciences) according to the manufacturer's protocol. The samples were acquired on a FACSCalibur flow cytometer (BD Biosciences) and analyzed with ModFit LT software version 4.0 or WinMDI version 2.9 (The Scripps Research Institute, La Jolla, CA, USA).

Transwell invasion assay. The invasive ability of cells was determined by transwell assay. Briefly, the 8-µm pore polycarbonate filters were coated with extracellular matrix (50 µg/filter; Sigma-Aldrich; Merck Millipore); 500 µl (2x104) cells were added to the upper chamber and 500 µl RPMI 1640 with 10% FCS was pipetted into the lower chamber. The non-invasive cells in the upper chamber were gently wiped off 12 h later. The cells that penetrated to the lower chamber were stained with crystal violet, imaged and counted. Each experiment was performed in triplicate.

Statistical analysis. All experiments were performed in triplicate at least. Data are expressed as the mean ± standard deviation. Statistical analyses were performed SPSS software version 13.0 (SPSS, Inc., Chicago, IL, USA). Groups were compared using one-way analyses of variance followed by the Student-Newman-Keuls post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Morphological alterations in A375 cells. Following culture with 12.5 mg/ml MENK for 48 h the A375 cells exhibited typical apoptotic morphology with long protrusions and

Table I. Methionine encephalin inhibits A375 cell invasion.

| Group     | Cell numbers | P-value |
|-----------|--------------|---------|
| Control   | 80.67±7.73   |         |
| 2.5 mg/ml | 79.33±8.21   | P=0.26  |
| 5 mg/ml   | 72.17±5.65   | P=0.001 |
| 10 mg/ml  | 64.67±5.01   | P<0.001 |
| 12.5 mg/ml| 57.83±3.31   | P<0.001 |
A375 cells exhibited disintegration and apoptotic bodies as presented in Fig. 1.

**MENK inhibits A375 cell proliferation.** The viability of A375 cells treated with MENK was reduced in a dose- and time-dependent manner. At 12.5 mg/ml MENK, the rate of cell growth inhibition reached a plateau; the rate of inhibition was 57.64 and 63.8% at 72 and 96 h, respectively, compared with the control group (P<0.001; Fig. 2A).

**MENK induces cell cycle arrest.** The effect of MENK on cell cycle distribution was analyzed by flow cytometry (Fig. 2B). The percentage of the 10 mg/ml MENK group in G0/G1 phase was 69.41% compared with 57.14% of the control cells (P=0.015). Correspondingly, the number of 10 mg/ml MENK group cells in S phase decreased to 14.8% compared to 29.52% of control cells (P=0.004). The percentage of cells subjected to 10 mg/ml MENK treatment in G2/M phase was 15.79% compared with 13.34% of control cells (P=0.24; Fig. 2C).

**MENK increases mRNA expression levels of opioid receptors on A375 cells.** Following treatment with 12.5 mg/ml MENK, mRNA expression levels of MOR, DOR and KOR on A375 cells were determined by RT-qPCR (Fig. 3). mRNA expression levels of DOR, MOR and KOR were 6.68, 5.46 and 2.81, respectively, compared with 1 in the control group. These differences reached significance for DOR (P<0.001) and MOR (P=0.001). This increased expression of all 3 ORs was attenuated by NTX (P=0.004).

**MENK induces apoptosis in vitro.** Following MENK treatment of A375 cells, decreased viability was accompanied by alterations in cell morphology, including the appearance of blebs and cell disintegration into apoptotic bodies. This was confirmed by annexin V/PI staining; there was a significant dose-dependent increase in annexin V+PI apoptotic cells following MENK treatment, compared with control cells (Fig. 4). Apoptosis appeared to peak at 48 h. The rate of apoptosis increased from 4.22% in the control group to 25.1% in the 12.5 mg/ml MENK treatment group (P=0.001).

**Discussion**

MENK, an endogenous neuropeptide, may contribute to immune responses against tumors and viral infections by activating multiple types of immune cells, enabling them to secrete various cytokines or directly kill target cells (19,20). Previous studies have provided evidence for the direct modulation of the immune system via opioid receptors (21,22). Investigations into MENK have revealed that the nuclear membrane of certain cancer cells expressed receptors to which MENK bound, resulting in marked growth inhibition of cancer cells in vitro (23-28).

The present study investigated the detailed mechanisms underlying the effects of MENK on human melanoma A375 cell growth. The results of the present study revealed that the concentration of MENK that achieved optimal inhibition of A375 cell growth, of those assessed, was 12.5 mg/ml. In addition, the mRNA expression levels of DOR, MOR and KOR in A375 cells increased following MENK treatment; DOR and MOR were predominant. This increased expression was attenuated by NTX. Furthermore, MENK induced apoptosis of A375 cells and inhibited invasion.

The inhibitory effects of MENK on cell replication were first described in developing rat brain (29) and in tissue culture...
studies on mouse and human neuroblastoma (30). MENK inhibits DNA synthesis and replication of healthy cells and tissues (31), human neoplasia and bacteria. The activity of MENK is receptor-mediated, dose- and time-dependent, and reversible. This neuropeptide is present in developing and regenerating tissues, and has been identified in embryonic tissues and numerous human cancers (32). Exogenous MENK inhibited progression and growth of a tumor overexpressing opioid receptors (33).

The present study demonstrated that MENK inhibited proliferation and induced apoptosis in A375 human melanoma cells for, to the best of our knowledge, the first time. Cells exhibited growth inhibition following exposure to MENK and MENK arrested cell cycle progression in G0-G1 phase. The action of MENK may therefore be closely associated with another key regulator of the G1-S phase transition, the tumor suppressor retinoblastoma protein. The effect of MENK on the cell cycle may be associated with opioid receptors; this is consistent with the study by Zagon et al (17), indicating the specific and singular receptor for MENK action on the replication of a human pancreatic cancer cell line is MOR.

MENK significantly increased apoptosis in A375 cells; XIAP and survivin were differentially expressed between control and MENK-treated groups. XIAP and survivin are members of the inhibitor of apoptosis (IAP) family, which are the most powerful apoptosis inhibitors currently identified. Survivin is important for the regulation of cell mitosis and apoptosis and is a dual-function protein, affecting the mitotic apparatus assembly and cytokinesis time, and regulating apoptosis through phosphorylation. Survivin is a mediator of apoptosis resistance and cell cycle progression, and is highly expressed in cancer. A previous study have revealed that survivin is upregulated in melanoma compared with healthy melanocytes, and is required for melanoma cell viability (34). A previous study in melanoma cell lines and animal models have implicated survivin as an important molecule in melanoma pathogenesis (35). Its clinical relevance in this disease is underscored by studies demonstrating a correlation between survivin expression and poor outcome in patients (36,37). The role of p53 in tumor suppression has been well characterized, acting in a gene-specific manner as a transcriptional activator (for pro-apoptotic genes including bax) or repressor (for various oncogenes including c-myc) (38). Induction of wild type p53 by DNA-damaging agents, including UV light and doxorubicin,
or introduction of exogenous p53 (39), downregulated survivin expression in malignant cells. In the present study, the expression of survivin was 0.011-fold in the MENK group compared with the control group at 48 h, suggesting that MENK may induce apoptosis by inhibiting survivin expression.

In conclusion, the results of the present study revealed, to the best of our knowledge for the first time, that MENK may inhibit growth and induce apoptosis of A375 cells, and describes a potential mechanism underlying these effects. MENK is readily degraded, without alteration of cell migration, differentiation or survival and thus may be considered a biotherapeutic agent. Clinically, MENK is safe, non-toxic and extends survival and reduces tumor burden in patients with unresectable pancreatic cancer (40). Therefore, MENK should be investigated as a primary therapy for human melanoma cancer and as an adjuvant to other chemotherapies. Further studies are required to develop

---

Figure 4. MENK induces apoptosis in A375 cells. (A) Annexin V/PI staining of A375 cells was performed following treatment with 0, 2.5, 5, 10 or 12.5 mg/ml MENK for 12, 24, 48 or 72 h. Apoptotic cells were defined as annexin V+/PI−. Experiments were performed 3 times with similar results and one representative experiment is presented. (B) The rate of apoptosis increased from 4.22% in the control group to 25.1% in the 12.5 mg/ml MENK treatment group. *P<0.05 and **P<0.01 vs. RPMI 1640. MENK, methionine encephalin; PI, propidium iodide.
an optimal strategy for the use of MENK in the treatment of human cancers.

Acknowledgements

The present study was supported by the China Liaoning Province Supporting Construction of Discipline Platforms in Universities and the China Liaoning Science Foundation (grant nos. 2009225008-7 and 2012225016). The authors thank other colleagues who contributed to the present study.

References

1. Miller AJ and Mihm MC Jr: Melanoma. N Engl J Med 355: 51-65, 2006.
2. Linos E, Swetter SM, Cockburn MG, Colditz GA and Clarke CA: Increasing burden of melanoma in the United States. J Invest Dermatol 129: 1666-1674, 2009.

3. Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, Altekruse SF, Kariyawasam S, Ruhl J, Tatalovich Z, et al. (eds): SEER Cancer Statfacts, 1975-2009 (Vintage 2009 Populations). http://seer.cancer.gov/csr/1975_2009_pops09/ accessed on October, 31 2013.

4. Bedikian AY, Millward M, Pehamberger H, Conry R, Gore M, Tuzer F, Pavlick AC, DeConti R, Hersh EM, Hersey P, et al.: Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: The Oblimersen Melanoma Study Group. J Clin Oncol 24: 4738-4345, 2006.

5. Engelmann JA and Jänne PA: Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res 14: 2895-2899, 2008.

6. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG and Varmus H: Acquired resistance of lung adenocarcinoma to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2: e73, 2005.

7. Hunt KJ, Hung SK and Ernst E: Botanical extracts as anti-aging preparations for the skin: A systematic review. Drugs Aging 27: 973-985, 2010.

8. Antignac E, Nohynek GJ, Re T, Clouseau J and Toutain H: Safety of botanical ingredients in personal care products. Cosmetics Food Toxicol 49: 324-341, 2011.

9. Reuter J, Merfort I and Schempp CM: Botanicals in dermatology: An evidence-based review. Am J Clin Dermatol 11: 247-267, 2010.

10. Baumann L, Woolery-Lloyd H and Friedman A: ‘Natural’ ingredients in cosmetic dermatology. J Drugs Dermatol 8: (Suppl 6) s5-s9, 2009.

11. Donahue RN, McLaughlin PJ and Zagon IS: Cell proliferation of human ovarian cancer is regulated by the opioid growth factor-opioid growth factor receptor axis. Am J Physiol Regul Integr Comp Physiol 296: R1716-R1725, 2009.

12. Vugj V, Stampic S and Dimitrijevic M: Methionine-enkephalin stimulates hydrogen peroxide and nitric oxide production in rat peritoneal macrophages: Interaction of mu, delta and kappa opioid receptors. Neuroimmunomodulation 11: 392-403, 2004.

13. Avella DM, Kimchi ET, Donahue RN, Tagaram HK, McLaughlin PJ, Zagon IS and Stavely-O’Carroll KF: The opioid growth factor-opioid growth factor receptor axis regulates cell proliferation of human hepatocellular cancer. Am J Physiol Regul Integr Comp Physiol 298: R549-R5466, 2010.

14. Zagon IS, Verderame MF and McLaughlin PJ: The biology of the opioid growth factor receptor (OGFr). Brain Res Brain Res Rev 38: 351-76, 2002.

15. Zagon IS and McLaughlin PJ: Targeting opioidergic pathways as a novel biological treatment for advanced pancreatic cancer. Expert Rev Gastroenterol Hepatol 6: 133-135, 2012.

16. Gredicak M, Supek F, Kralj M, Majer Z, Hollosi M, Smuc T, Mlinarić-Majerski K and Horvat S: Computational structure-activity study directs synthesis of novel antitumor agents. Amino Acids 38: 1185-1191, 2010.

17. Zagon IS, Roseneu CD, Verderame MF, Ohlsson-Wilhelm BM, Levin RJ and McLaughlin PJ: Opioid growth factor regulates the cell cycle of human neoplasias. Int J Oncol 17: 1053-1061, 2000.

18. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.

19. DeBenedette MA, Calderhead DM, Ketteringham H, Gamble AH, Horvatovinich JM, Tchepepanova JY, Niecolette CA and Healey DG: Priming of a novel subset of CD28² rapidly expanding high-avidity effector memory CTL by post maturation electroporation-CD40L dendritic cells is IL-12 dependent. J Immunol 181: 5296-5305, 2008.

20. Johnson AJ, Mendez-Fernandez Y, Moyer AM, Sloma CR, Pirko I, Block MS, Rodriguez M and Pease LR: Antigen-specific CD8+ T cells mediate a peptide-induced fatal syndrome. J Immunol 174: 6854-6862, 2005.

21. Carr DJ and Carpenter GW: Morphine-induced suppression of cytotoxic T cell activity in allogeneically exposed mice is not mediated through a naltirindole-sensitive delta opioid receptor. Neuroimmunomodulation 2: 44-53, 1995.

22. Carr DJ, Carpenter GW, Garza HH Jr, Baker ML and Gebhardt BM: Cellular mechanisms involved in morphine-mediated suppression of CTL activity. Adv Exp Med Biol 573: 131-139, 1995.

23. Zagon IS, Verderame MF, Hankingo J and McLaughlin PJ: Opioid expression of the opioid growth factor receptor potentiates growth inhibition in human pancreatic cancer cells. Int J Oncol 30: 775-83, 2007.

24. Zagon IS, Donahue RN and McLaughlin PJ: Opioid growth factor-opioid growth factor receptor axis is a physiological determinant of cell proliferation in diverse human cancers. Am J Physiol Regul Integr Comp Physiol 297: 1154-1161, 2009.

25. Zagon IS, Kreiner S, Heslop JJ, Conway AB, Morgan CR and McLaughlin PJ: Prevention and delay in progression of human pancreatic cancer by stable overexpression of the opioid growth factor receptor. Int J Oncol 33: 317-323, 2008.

26. Goldenberg D, Zagon IS, Fedok F, Cist HS and McLaughlin PJ: Expression of opioid growth factor (OGF)-OGF receptor (OGFr) axis in human nonmedullary thyroid cancer. Thyroid 18: 1165-1170, 2008.

27. McLaughlin PJ, Kreiner S, Morgan CR and Zagon IS: Prevention and delay in progression of human squamous cell carcinoma of the head and neck in nude mice by stable overexpression of the opioid growth factor receptor. Int J Oncol 33: 751-757, 2008.

28. Smith JP, Bingman SI, Mauger DT, Harvey HH, Demers LM and Zagon IS: Opioid growth factor improves clinical benefit and survival in patients with advanced pancreatic cancer. Open Access J Clin Trials 2010: 37-48, 2010.

29. Wu Y, McLaughlin PJ and Zagon IS: Ontogeny of the opioid growth factor, [Met5]-enkephalin, preproenkephalin gene expression, and the zeta opioid receptor in the developing and adult animal. Dev Dyn 231: 327-338, 2005.

30. Zagon IS, Ruth TB and McLaughlin PJ: Nucleocytoplasmic distribution of opioid growth factor and its receptor and its receptor in tongue epithelium. Anat Rec A Discov Mol Cell Evol Biol 282: 24-37, 2005.

31. McLaughlin PJ, Levin RJ and Zagon IS: Regulation of human head and neck squamous cell carcinoma growth in tissue culture by opioid growth factor. Int J Oncol 14: 991-998, 1999.

32. Zagon IS, Porterfield NK and McLaughlin PJ: Opioid growth factor-opioid growth factor receptor axis inhibits proliferation of triple negative breast cancer. Exp Biol Med (Maywood) 238: 589-599, 2013.

33. Tsanova A, Jordanova A, Dzimbova T, Pujoinova T, Golovinsky E and Lalchev Z: Interaction of methionine-enkephalins with raft-forming lipids: Monolayers and BAM experiments. Amino Acids 46: 1159-1168, 2014.

34. Liu T, Brouha B and Grossman D: Rapid induction of mitochondrial events and caspase-independent apoptosis in Survivin-targeted melanoma cells. Oncogene 23: 39-48, 2004.

35. Yang J, Wahdan-Alaswad R and Danielpour D: Critical role of Smad2 in tumor suppression and transforming growth factor-beta-induced apoptosis of prostate epithelial cells. Cancer Res 69: 2185-2190, 2009.

36. Song K, Krebs TL and Danielpour D: Novel permissive role of epidermal growth factor in transforming growth factor beta (TGF-beta) signaling and growth suppression. Mediation by stabilization of TGF-beta receptor type II. J Biol Chem 281: 7765-7774, 2006.

37. Shehata HH, Abou Ghalia AH, Elsayed EK, Ziko OO and Mohamed SS: Detection of Survivin protein in aqueous humor and serum of retinoblastoma patients and its clinical significance. Clin Biochem 43: 362-366, 2010.

38. Tang L, Ling X, Liu W, Das GM and Li F: Transcriptional inhibition of p21WAF1/CIP1 gene (CDKN1) expression by survivin is at least partially p53-dependent: Evidence for survivin acting as a transcription factor or co-factor. Biochem Biophys Res Commun 421: 249-254, 2012.

39. Song K, Cornelius SC and Danielpour D: Development and characterization of DP-153, a nonmutogenic prostatic cell line that undergoes malignant transformation by expression of dominant-negative transforming growth factor beta receptor type II. Cancer Res 63: 4358-4367, 2003.

40. Zagon IS and McLaughlin PJ: Opioid growth factor and the treatment of human pancreatic cancer: A review. World J Gastroenterol 20: 2218-2223, 2014.