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Suppression of lymphangiogenesis in human lymphatic endothelial cells by simultaneously blocking VEGF-C and -D/VEGFR-3 with norcantharidin

Zhong-Yan Liu[1*], Hai-Ou Qiu[2*], Xiao-Jian Yuan[1*], Yan-Yun Ni[1], Jian-Jun Sun[1], Wei Jing[1], Yue-Zu Fan[1]
[1]Department of Surgery, [2]Department of Otolaryngology, Tongji Hospital, Tongji University School of Medicine, Shanghai, P.R. China
*Contributed equally

Lymph node metastasis of tumor could be a crucial early step in the metastatic process. Tumor lymphangiogenesis may play an important role in promoting tumor metastasis to regional lymph nodes. Norcantharidin (NCTD) has been reported to possess potent antiangiogenesis and antitumor properties in several cell lines and xenograft tumor models. However, its role in tumor-associated lymphangiogenesis and lymphatic metastasis remains unclear. Here, we investigated the effect of NCTD on proliferation, apoptosis, migration, invasion, lymphatic tube-formation, i.e. lymphangiogenesis of human lymphatic endothelial cells (HLECs) *in vitro* by MTT, proliferation assay, Hoechst staining and flow cytometry, scraping line method, Matrigel invasion assay, inverted or fluorescence microscope and transmission electron microscope. Moreover, the underlying mechanisms, such as vascular endothelial growth factor-C (VEGF-C), -D (VEGF-D), -receptor 3 (VEGFR-3) at protein and mRNA levels in lymphangiogenesis on 3-dimensional (3-D) culture of HLECs were measured by immunohistochemistry, Western-blot and real-time polymerase chain reaction (RT-PCR). It was shown that NCTD inhibited HLECs' proliferation, migration, invasion and lymphatic tube formation (forming-lymphatic and/or formed-lymphatic), induced HLECs' apoptosis (all $P<0.01$) significantly, with dose- and time-dependent (IC_{50} 6.8 μg/ml); and downregulated the expression of VEGF-C, VEGF-D and VEGFR-3 at protein or/and mRNA levels ($P<0.01$) in HLECs' lymphatic tube-formation. Thus, we identified for the first time that NCTD inhibited HLECs' lymphangiogenesis by simultaneously blocking VEGF-C and -D/VEGFR-3 *in vitro*. The present findings may be of importance to explore the therapeutical target or strategy of NCTD for tumor lymphangiogenesis and lymphatic metastasis.

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Crosstalk between TLR-4 and Wnt/beta-catenin signaling during intestinal chemotherapy-induced mucositis

Igor Sukhotnik
The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Laboratory of intestinal adaptation and recovery, Dept of Pediatric Surgery, Bnai Zion Medical Center, Haifa, Israel

Toll-like receptor 4 (TLR-4) and Wnt/β-catenin signalling are crucial in maintaining intestinal epithelial homeostasis, participates in a vigorous signaling process. The objective of this study was to determine the effects of methotrexate (MTX) and glutamine (GLN) on TLR-4 and Wnt/β-catenin signalling in intestinal mucosa during chemotherapy-induced mucositis in a rat. Male Sprague-Dawley rats were randomly assigned to one of four experimental groups: 1) control rats; 2) CONTR-GLN animals were treated with oral glutamine given in drinking water (2%) 48 hours before and 72 hours following vehicle injection; 3) MTX-rats were treated with a single IP injection of MTX (20mg/kg); and 4) MTX-GLN were pre-treated with oral glutamine given similar to group B. Intestinal mucosal damage, mucosal structural changes, enterocyte proliferation and enterocyte apoptosis were determined 72 hours following MTX injection. The expression of TLR-4, MyD88, TRAF6, WNT3A, β-catenin in the intestinal mucosa was determined using Real time, Western blot and immunohistochemistry. MTX-induced mucositis resulats in a significant increase in intestinal mucosal injury score and decreased cell proliferation. MTX-GLN rats demonstrated a greater jejunal and ileal mucosal weight, greater villus height and crypt depth and index of proliferation in jejunum and ileum, compared to MTX animals. MTX-induced mucositis resulted in a significant down-regulation of TLR-4, MyD88, TRAF6 and concomitant increase in β-catenin mRNA and protein levels. The administration of GLN increased significantly the expression of TLR-4 and MyD88 (vs MTX group). In conclusion, MTX-induced mucositis is associated with inhibition of TLR-4 signalling. Treatment with glutamine attenuates the inhibitory effect of MTX on TLR-4 and MyD88 expression and concomitant decrease in intestinal mucosal injury caused by MTX-induced mucositis.

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Parthenolide induces caspase-independent and AIF-mediated cell death in tumor cell lines

S. Emanuele, A. D'Anneo, D. Carlisi, M. Lauricella, R. Martinez, R. Vento and G. Tesoriere
Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche (BioNec); Sezione di Scienze Biochimiche, Policlinico, via del Vespro 129, 90127 Palermo, Italy

Parthenolide (PN), a sesquiterpene lactone extracted from Tanacetum parthenium, has recently attracted considerable attention for its complex pharmacological action including anti-inflammatory and anti-cancer effects. We have preliminarily analysed the effect of PN on a number of tumor cell lines. Then our study was focused on human MG-63 osteosarcoma and SK-Mel 28 melanoma cells, two cell lines on which PN exerted a similar action mechanism. In the first phase of treatment (0-5 h) of both the cell lines PN induced chromatin condensation and fragmentation and nuclear shrinkage whereas in the second phase of treatment (5-15 h) necrotic signs became progressively predominant. Interestingly, the PN effect was independent of caspases in both the cell lines since the pan-caspase inhibitor z-VAD did not protect the cells from death. In addition, PN determined a marked decrease in the level of pro-caspase 8 and pro-caspase 3 which was not accompanied by caspase activation. Treatment with PN rapidly stimulated ROS generation, an event which mainly depended on NADPH oxidase and successively on the mitochondria. ROS generation caused depletion of thiol groups and GSH, NF-κB inhibition, and JNK activation. Oxidative stress, together with the mitochondrial accumulation of Ca^{2+}, also favoured dissipation of $\Delta\psi m$, which seemed primarily determined by PTP opening. Considering the involvement of mitochondria, we focused on the caspase-independent apoptosis inducing factor (AIF). Our results demonstrated that in both the cell lines PN caused the translocation of AIF from the mitochondria to the nuclei. Because down-regulation of AIF by siRNA in both the cell lines markedly inhibited the PN effects on chromatin condensation and cell necrosis, we conclude that AIF plays a crucial role in the PN action.

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microRNA-dependent targeting of Syndecan-1 modulates cancer stem cell properties and invasiveness in breast cancer

Sherif A. Ibrahim[1,2], Hebatallah Hassan[1,2], Claudia Neubauer[1], Ludwig Kiesel[1], George W. Yip[3], Burkhard Greve[4], Martin Götte[1]
[1]Department of Gynecology and Obstetrics, Münster University Hospital, Münster, Germany; [2]Department of Zoology, Faculty of Science, Cairo University, Giza, Egypt; [3]Department of Anatomy, National University of Singapore, Singapore; [4]Department of Radiotherapy, University Hospital, Münster, Germany
E-mail: mgotte@uni-muenster.de

microRNAs are small endogenous noncoding RNAs, which post-transcriptionally regulate gene expression. In breast cancer, aberrant expression of the transmembrane heparan sulfate proteoglycan syndecan-1 correlates with poor clinical outcome (1). In this study, we identify syndecan-1 as a novel target for the oncomiR miR-10b (2), as demonstrated by 3'UTR-luciferase assays, qPCR and FACS analysis. siRNA-mediated knockdown of syndecan-1 and overexpression of miR-10b concordantly promoted matrigel chamber invasiveness, and migration of MDA-MB-231 breast cancer cells. Affymetrix screening and confirmatory qPCR and Western blotting analysis revealed that altered focal adhesion kinase activation associated with differential expression of the transcription factors ATF2 and RUNX1 resulted in a proinvasive downregulation of E-cadherin and dysregulated Rho-GTPase function in syndecan-1-depleted cells. As previous observations had demonstrated an altered wnt1-responsive precursor pool and resistance to experimentally induced breast cancer in syndecan-1-deficient mice (3), we studied potential changes in the stem cell phenotype of MDA-MB-231 and MCF-7 breast cancer cells. FACS analysis revealed that syndecan-1 siRNA-depletion resulted in a reduction of the CD44+ CD24- breast cancer stem cell pool and in a reduced ALDH1 activity. Our data reveal a dual function of syndecan-1 in breast cancer: Syndecan-1 promotes the cancer stem cell phenotype, while it inhibits invasiveness of breast cancer cells, with implications for the mode of syndecan-1 targeting in a clinical setting.

(1) Loussouarn D et al. Br J Cancer 98:1993-8 (2008)
(2) Ma L et al. Nature 449:682-8 (2007)
(3) McDermott SP et al. Oncogene 26:1407-16 (2007)