Recent molecular and clinical studies have shown that invasion and metastasis may occur very early in tumour development, thus emphasizing the potential importance of spesific and sensitive detection of circulating tumour cells (CTC). With the ability of refined technologies the identification of CTCs from peripheral blood is emerging as a useful tool for the detection of malignancy, monitoring disease progression and measuring response to therapy.
The goal of this study was to identify optimal marker or marker combinations for detection of CTCs in the gastrointestinal malignancies using RT-PCR.
Materials and Methods: To detect the presence of CTCs, we analyzed Cytokeratin 19 (CK19), Cytokeratin 20 (CK20) and Mucin 1 (MUC1) mRNA in the peripheral blood of 31 patients with gastrointestinal (gastric, stomach and colorectal) carcinoma and 30 healthy individuals.
Results: In RT-PCR analysis of the peripheral blood, 77.4% (24/31), 58.06% (18/31) and 3.22% (1/31) of cancer patients were positive for MUC1, CK20 and CK19 mRNA respectively. The sensivity and specificity for any one of mRNA detected in peripheral blood is 83.3% and 66.6% respectively, with an acurracy of 59%.
Conclusions: Our study suggest that MUC1 and CK20 mRNAs in the peripheral blood could be useful molecular markers for gastrointestinal tumours. Combination of these two tumour-spesific mRNA markers would increase the detection rate and may be clinically helpful in predicting the tumour presence and colorectal cancer metastasis.

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Docosahexaenoic Acid Induces Cell Death Through ROS-dependent ERK and JNK Activation in Human Ovarian Cancer Cells

K. Lim[1], S. Jeong[1], K.S. Song[1], N. Kim[1], K. Jing[1], S. Shin[1], H. Oh[1], J.I. Park[1], W.H. Yoon[1], B.D. Hwang[1]. [1]*Chungnam National University Medical School, Biochemistry, Daejeon, Korea*

Background: Ovarian cancer is the main cause of cancer death from gynecologic tumours. Although there are abundant experimental evidences showing that the ω3-polyunsaturated fatty acids (ω3-PUFAs) rich in fish oil, such as DHA and EPA, inhibit cancer development and growth, the molecular mechanisms on the anti-cancer actions of ω3-PUFAs remain incompletely understood. In the present study, anticancer action of docosahexaenoic acid (DHA) has been investigated in ovarian cancer cells.
Material and Methods: The effects of DHA on cell proliferation and cell cycle were examined by MTT assay and FACS. DHA-induced apoptosis was analyzed using the TUNEL assay, caspase activity assay, and western blot. Dihydroethidium (DHE) was used in PA-1cells for reactive oxygen species (ROS) measurement.
Results: DHA induced cell cytotoxicity in ovarian cancer cell lines including PA-1, MDAH2774 and ID8. Following treatment of DHA, the cell proliferation of PA-1 cells was decreased in a dose and time-dependent manner. Further study showed that the DHA-induced cytotoxicity was mainly associated with apoptosis as caspase 3 activity, TUNEL-positive cells and the portion of sub-G1 cells were significantly increased in the cells treated with DHA. DHA dramatically increased cellular level of phospho-ERK and phospho-JNK Pretreatment of ERK inhibitor, U0126, partially protected the cell death caused by DHA, indicating protective role of ERK activation in DHA-induced cell death. In addition, an oxidative process was implicated in apoptosis induced by DHA since DHA increased ROS level in PA-1 cells. ROS scavenger, NAC, prevented DHA-increased ROS production and cytotoxicity. Finally, pretreatment of PA-1 cells with NAC attenuated ERK and JNK activation and cell death induced by DHA.
Conclusions: DHA induces cell death through ROS-dependent ERK and JNK activation in human ovarian cancer cells, thus providing important evidence and molecular insights for the use of ω3-PUFAs in chemoprevention and treatment of ovarian cancer.
This work was supported by basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (2010–0001290).

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Microtubule − a Target of Withaferin-A Induced Cell Death

K. Ghosh[1], A. Das[2], A. Lahiri[1], G. Chakraborti[2], S. Sengupta[1]. [1]*University of Calcutta, Biophysics Molecular Biology and Genetics, Calcutta,* [2]*University of Calcutta, Biotechnology, Calcutta, India*

Background: The tubulin-microtubule equilibrium in a cell has been a very popular target for anticancer therapy; while paclitaxel and vinblastine are two widely used drugs employed for treating cancer, many natural products are getting importance in this field of search. Withaferin-A (WA), derived from Withania-somnifera-Dunal, induces cell cycle arrest and apoptosis in different carcinomas. WA alters cytoskeletal structure by targeting actin microfilament, as well as intermediate filament protein vimentin. Heretofore, no evidence has been reported regarding its effect on microtubule assembly. This study is to elucidate the mechanism of inhibiting microtubule assembly by WA.
Methods: The IC-50 value of WA was determined by MTT assay, apoptosis and cell cycle assay was performed by FACS analysis, wound healing assay was done to see cellular migration, immune fluorescent detection was done to check microtubular network of WA treated cells. Effect of WA on tubulin polymerization in vitro was studied by light scattering assay, binding measurements of WA to tubulin was determined by flurometric assay and probable WA-tubulin interaction site was proposed by molecular modeling method.
Results: We found, WA inhibited proliferation of MCF7, A549 and HeLa cells by inducing apoptosis in concert with cell cycle arrest in different phases, effectively inhibited cellular migration, caused significant disruption of interphase and spindle microtubular organization. WA inhibits microtubule polymerization of purified goat brain tubulin *in vitro*. Binding of WA to tubulin quenches tryptophan fluorescence of tubulin, alters fluorescence of ANS-tubulin complex and stiochiometry of WA is to tubulin binding was 1:1 (molar ratio) with a dissociation constant of $14.30 \pm 5.35\ \mu M$. Competition assay showed no binding of WA to colchicine binding site of tubulin. Molecular docking simulations indicated that WA preferentially binds to a novel site to tubulin.
Conclusion: It is evident that WA suppresses microtubule dynamics by directly binding to tubulin which sheds light on mechanisms behind its anti-proliferative activity.

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Role of the E2F1 Transcription Factor in Transforming Growth Factor-β-mediated Apoptosis

J. Korah[1], N. Falah[1], J.J. Lebrun[1]. [1]*McGill University Health Centre, Department of Medicine, Montreal, Canada*

Tumour formation is linked to a series of well-defined events that occur in most human cancers, including the ability of cells to attain immortalization and to evade apoptosis. Transforming growth factor-β (TGFβ) exerts its tumour suppressive effects by inhibiting cell growth, preventing immortalization through inhibition of telomerase, and inducing apoptosis. In a previous study, we showed that TGFβ-mediated inhibition of telomerase requires an intermediate molecule that we identified as the transcription factor E2F1. The E2F family of transcription factors plays a central role in regulating cell-cycle progression, and deregulation of these factors is a common event in most human cancers. The transcriptional activity of E2F1 is regulated primarily via its association with the retinoblastoma tumour suppressor protein, pRb. Interestingly, E2F1 has been shown to induce both cell cycle progression and apoptosis, though the mechanisms of E2F-mediated apoptosis have not been fully elucidated. As TGFβ itself is a potent proapoptotic factor, we investigated the potential implication of E2F1 in TGFβ-mediated apoptosis.
Expression analysis by Western blot demonstrated that E2F1 itself is upregulated by TGFβ in a number of varying cancer types and cell lines. Transient siRNA knockdown of the Smad proteins indicated that these canonical downstream effectors of TGFβ signalling are implicated in this regulation. Analysis by quantitative real-time PCR indicated that numerous proapoptotic genes known to be induced by E2F1 are also regulated by TGFβ and, in particular, we identified Smac/DIABLO as a novel TGFβ target. In addition to regulating specific apoptotic genes, TGFβ reduced cell viability and induced apoptosis in a number of cell lines, as assessed by MTT and Calcein-AM viability assays as well as AnnexinV staining and PARP cleavage. Importantly, transient siRNA knockdown experiments indicated that loss of E2F1 antagonized these TGFβ proapoptotic effects. Overexpression experiments with dominant negative E2F1 mutants revealed that pRb binding to E2F1 is implicated in TGFβ-mediated apoptosis. Immunoprecipitation studies confirmed that TGFβ induces association of E2F1 and pRb and additionally revealed the recruitment of the histone acetyltransferase P/CAF to this complex in response to TGFβ. Moreover, chromatin immunoprecipitation (ChIP) analysis indicated that this E2F1-pRb-P/CAF complex is recruited to the promoters of a number of proapoptotic genes in response to TGFβ.
These data strongly support a proapoptotic role for the E2F1 pathway downstream of TGFβ and provide a potential mechanism for the TGFβ-mediated activation of E2F1-responsive proapoptotic genes. Together, our results highlight E2F1 as a critical regulator of TGFβ tumour suppressive effects; in addition to its role in mediating TGFβ-induced inhibition of telomerase, we now show that E2F1 is also central to TGFβ-mediated apoptosis.