
“Cell Death: Aging, Metabolism and Ramifications for Therapeutics and Drug Development.”

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Abstract:

AUTOPHAGY AS A MECHANISMS FOR SENSITIZATION OF BREAST CANCER CELLS TO DOXORUBICIN THERAPY. Thomas MP, Engelbrecht A-M*, Dept of Physiological Sciences, Stellenbosch University, Stellenbosch, South Africa

Cells are unable to store amino acids and must therefore draw them from local blood vessels. In microenvironments where this is not possible, endogenous promotion of autophagy may maintain intracellular amino acids levels so that they remain available for macromolecular biosynthesis and ATP production. We hypothesize that an increase in autophagy in response to amino acid deprivation will correlate with an elevated sensitivity of breast cancer cells to doxorubicin toxicity.

Two human breast cancer cell lines (MCF-7 and MDA-MB-231) are compared with a normal epithelial cell line (MCF-12A). Autophagy levels were measured by monitoring classical protein markers of autophagy (LC3-II and beclin-1) and acidic compartmentalization in cells (Lysotracker red dye), in conjunction with an inducer (rapamycin) and an inhibitor (bafilomycin A1) of autophagy.

Our results indicate that total amino acid deprivation leads to a time dependant increase in autophagy. Apoptotic cell death is diminished upon interference of autophagy, indicating its potential involvement in resistance to cell death during amino acid starvation. We demonstrate that doxorubicin promotes autophagy in a concentration and time dependent manner. High levels of doxorubicin cause a rapid, transient increase in autophagy while low concentrations increase autophagy only following relatively longer incubation times. We also present evidence that promotion of autophagy by total deprivation of amino acids for certain time periods causes greater levels of doxorubicin induced cell death. This novel approach to tumour sensitization could have several implications in the context of cancer therapy.
**Novel treatment strategies for cancer – talks of interest and relevant to our work:**

**Simvastatin-induced cell death in colorectal cancer – Zhu Yan, Dept of Physiology, School of Medicine, National University of Singapore.**

Statins are widely used as cholesterol-lowering drugs that selectively inhibit the enzyme, 3-hydroxy-3-methylglutaryl CoA reductase of the mevalonate biosynthetic pathway. Although recent studies suggested that statins possess anti-tumor properties, the underlying mechanisms are still not understood. Mevalonate or GGPP (geranylgeranylpyrophosphate) but not farnesylpyrophosphate (FPP) in combination with simvastatin prevented the cell death phenotype, suggesting the involvement of geranylgeralynated proteins instead of farnesylated proteins. Simvastatin also induced a significant increase in intracellular ROS production. Pre-incubation with ROS scavengers completely prevented simvastatin-mediated cell death. Simvastatin treatment activated the stress response via c-Jun NH2-terminal kinase (JNK) and up-regulated pro-apoptotic BIM expression, which was followed by mitochondrial cell death pathway – simvastatin-induced apoptosis in colorectal cancer cells is mediated by cross talk between intracellular ROS production and JNK-Bim signaling.

**Gamma-tocopherol and Lovastatin in programmed cell death induction in human colorectal carcinoma cells – Rezaei Mohsen – Department of Toxicology and Pharmacology, Faculty of Pharmacy, JundiShapur University of Medical Sciences, Ahvaz, Iran.**

HT29 cells were grown in DMEM and treated with different concentrations of lovastatin (10, 20, 40 100 μM) and gamma-tocopherol (25, 50, 100, 200 μM) for 48 and 72h individually and in combination. Disruption of mitochondrial membrane potential and phenotype of apoptosis (caspase-3 cleavage) was observed. Lovastatin in combination with gamma-tocopherol significantly enhanced the apoptotic phenotype resembling an additive or synergistic effect.
Topoisomerase II as a potential target in the anti-cancer activity of the novel small molecule compound C2 – Lee Soo Fern – Dept of Physiology, Yong Loo Lin School of Medicine, National University of Singapore.

Exposure of HCT116 cells to increasing concentration of compound C2 resulted in significant reduction in cell viability as well as inhibition of tumor colony formation. This growth inhibitory activity was independent of p53 expression, as results demonstrated pronounced G2/M cell cycle arrest in both p53WT and p53KO variants of the HCT116 cell line. Treatment of mice carrying HCT116 xenografts with repeat injections of compound C2 showed pronounced inhibition of tumor volume and growth. Compound C2 functioned as a potent topoisomerase II inhibitor, however it did not enhance the formation of the DNA cleavable complex – it was thus classified as a topoisomerase II catalytic inhibitor. Compound C2 did not block the ATPase activity of topoisomerase II, but instead it blocked the initial DNA incision process, thereby leading to the complete abrogation of topoisomerase II function.

Secreted frizzled-related protein 4 expression is positively associated with responsiveness to cisplatin of ovarian cancer cell lines in vitro and with lower tumour grade in mucinous ovarian cancers – Dharamarajan Arun – School of Anatomy, Physiology and Human Biology, Faculty of Life and Physical Sciences, University of Western Australia, Crawley, Australia.

Ovarian cancer is one of the most lethal malignancies in women, as it is frequently detected at an advanced stage when cancers often become refractory to chemotherapy. Evidence suggested that dysregulation of pro-apoptotic genes plays a key role in the onset of chemoresistance. The secreted Frizzled-Related Protein (sFRP) family is pro-apoptotic and also a negative modulator of the Wnt signaling cascade. Studies have demonstrated that re-expression of sFRPs, in particular sFRP4, is associated with better prognosis, and that experimentally induced expression results in apoptosis. In vitro experimental models determined that sFRP4 was differentially expressed between chemosensitive (A2780) and chemoresistant (A2780 ADR and A2780 Cis) cancerous ovarian cell lines, with
chemosensitive cells expressing significant higher levels of sFRP4. Transfection of the chemoresistant cell lines with sFRP4 significantly increased their sensitivity to chemotherapy. Conversely, silencing of sFRP4 expression in the chemosensitive cell line resulted in a corresponding increase in chemoresistance. Comparison of sFRP4 expression in tumour biopsies revealed a positive trend between sFRP expression and tumour grade, with malignant adenocarcinomas exhibiting significantly decreased sFRP4 levels compare to borderline tumours.

MicroRNA-mediated targeting of Hedgehog signaling pathway promotes apoptosis in drug-resistant CD34+ CML stem cells – Sadeghizadeh Majid – Dept of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

Although tyrosine kinase inhibitors designed to inhibit BCR-ABL oncogenes are used in the therapy of chronic-phase of chronic myeloid leukemia (CML), treatment efficacy is dramatically decreased due to the emergence of drug-resistant leukemic stem cell (LSC) clones in the blast crisis phase of the disease. Acquisition of self-renewal property due to aberrant activation of Hedgehog (Hh) signaling contributes to CML progression. Therefore, development of a molecular approach that specifically targets the LSC pool is essential to cure CML. The aims of this study were to detect microRNAs which are differentially expressed in CD34+ CML cells and the evaluation of their apoptotic effects following overexpression or downregulated microRNAs. CD34+ CML cells were isolated by using immunomagnetic separation system from bone marrow of blast crisis patients. MicroRNA expression levels were quantified with Q-RT-PCR. CD34+ CML stem cells were transduced with recombinant lentiviruses expressing microRNA of interest. The downregulated microRNA signature which was identified consists of miR-324-5p and miR-326 in CD34+ CML cells with high Hh signaling. Q-RT-PCR and western blot analysis showed that these microRNAs target the activator components of the pathway, Smo and Gli1, thereby inducing apoptosis and suppressing cell proliferation. Aberrant Hh pathway activation is a feature of chemotherapy drug-resistant CD34+ CML cells. Hh inhibition via upregulation of microRNAs differentially expressed between
CML and normal CD34+ cells induced apoptosis. This highlights the necessity of inhibition of other targets besides BCR-ABL, like Hh signaling that regulates leukemic self-renewal, in order to eradicate the BCR-ABL positive CD34+ LSCs. This targeting approach may serve as a potential therapeutic strategy for curing CML.

Identification of gamma-tocotrienol as a novel chemosensitizer in a xenograft mouse model of human gastric cancer – Gautam Sethi – Dept of Pharmacology, Yong Loo Lin School of Medicine, Cancer Science Institute, National University of Singapore.

Gamma-tocotrienol, a member of the Vitamin E superfamily has attracted attention for its anti-proliferative and anti-carcinogenic potential against many different cancers. The anti-proliferative and chemosensitizing effects of γ-tocotrienol are associated with its ability to suppress activation of signal transducers and activator of transcription 3 (STAT3), a pro-inflammatory transcription factor that plays a pivotal role in the survival, proliferation, angiogenesis and chemoresistance of hepatocellular carcinoma. However, the potential of Y-tocotrienol to overcome chemoresistance in gastric cancer, which is the deadliest cancer in Asia-pacific region, has not been explored. The effect of γ-tocotrienol in combination with capecitabine to modulate tumor growth and survival was evaluated in a xenograft mouse model. Cell proliferation and apoptosis assays indicated that γ-tocotrienol potentiated capecitabine induced programmed cell death in various gastric cell lines. It also inhibited expression of BCI-2, Bcl-XL, cyclin D1, COX-2, ICAM-1, VEGF, CXCR4, MMP-9 proteins, induced PARP cleavage and inhibited constitutive and capecitabine-induced NF-κB activation in gastric cancer cells. In vivo studies using a xenograft model of human gastric cancer demonstrated that γ-tocotrienol alone suppressed tumor growth and this effect was further potentiated in conjunction with capecitabine. Markers of proliferation index Ki-67 and the micro vessel density CD31 were significantly downregulated in tumor tissues by the combination therapy. As compared to the vehicle control, γ-tocotrienol also suppressed NF-κB activation and
expression of cyclinD1, COX-2, ICAM-1, MMP-9 and survivin in tumor tissues obtained from treatment groups. γ-Tocotrienol can thus potentiate the effects of capecitabine through modulation of multiple markers of proliferation, invasion, angiogenesis, and metastasis in gastric cancer.

Lung cancer: mechanisms of resistance and sensitivity to treatment – Boris Zhivotovsky – Institute of Environmental Medicine, Division of Toxicology, Karolinska Institute, Stockholm, Sweden.

Lung cancer

Small cell lung carcinoma (SCLC): 15-20%
Non-small cell lung carcinoma (NSCLC): 75%
  - Adenocarcinoma (30%)
  - Squamous cell carcinoma (30%)
  - Large cell carcinoma (5-10%)

SCLC is characterized by relatively high sensitivity to treatment with anticancer drugs and radiation. However, despite the initial responsiveness, relapses occur in most cases and are accompanied by fast resistance to treatment. SCLC represents a highly malignant and particularly aggressive form of cancer with early and widespread metastasis and poor prognosis. Because of early dissemination, this type of tumor cannot be surgically removed. If the disease is local, concomitant chemosensitivity and radiotherapy can be curative. Unfortunately, only 10% of SCLC patients present with localized disease, therefore the search for new approaches to cure SCLC is essential. Approximately 80% of all SCLC cell lines and tumors do not express caspase-8 because hypermethylation or mutations in the CASP8 gene. Irrespective of expression of this protein, almost all SCLC are resistant to TRAIL monotherapy. The presenter discussed several ways which was discovered in their lab to sensitize SCLC cells, expressing or lacking caspase-8, to TRAIL. In contrast to SCLC, NSCLC at stage I and II can be removed surgically. However, because of late diagnosis, the majority of cases are only recognized at stage III and is characterized by high resistance to
both chemo- and radiotherapy, and complete remission upon therapy is rare. Resistance to cytotoxic drugs and γ-radiation is associated with dysfunction of the mitochondria. Furthermore, PKC inhibitors can reactivate the full apoptotic response in NSCLC via ROS generation and increased intracellular Ca\(^{2+}\) concentration.

**Metabolic vulnerabilities and cancer cell death – David Hockenbery – Fred Hutchinson Cancer Research Center, Seattle, USA.**

Cell proliferation requires the coordinated activity of cytosolic and mitochondrial metabolic pathways to provide ATP and building blocks for DNA, RNA, and protein synthesis. Many metabolic pathway genes are targets of the c-myc oncogene and cell cycle regulator. However, the contribution of c-Myc to the activation of cytosolic and mitochondrial metabolic networks during cell cycle entry is unknown. The presenter reported the metabolic fates of \([U-^{13}C]\) glucose in serum-stimulated \(myc^{-/-}\) and \(myc^{+/+}\) fibroblasts by \(^{13}\)C isotopomer NMR analysis. They demonstrated that endogenous c-myc increased \(^{13}\)C-labeling of ribose sugars, purines, and amino acids, indicating partitioning of glucose carbons into C1/folate and pentose phosphate pathways, and increased tricarboxylic acid cycle turnover at the expense of anaplerotic flux. Myc expression also increased global O-linked GlcNAc protein modification, and inhibition of hexosamine biosynthesis selectively reduced growth of Myc-expressing cells, suggesting its importance in Myc-induced proliferation. Their data revealed a central organizing role for the Myc oncogene in the metabolism of cycling cells. The pervasive deregulation of this oncogene in human cancers may be explained by its role in directing metabolic networks required for cell proliferation.
Oncogene driven redox cell survival mechanisms – Thomas Cotter – Dept of Biochemistry, University College Cork, Ireland.

Reactive oxygen species (ROS) are a group of molecules produced in the cell through metabolism of oxygen. They have well-established roles in generating genomic instability in cancer cells which has led to the characterization of these molecules as non-specific agents of destruction. It is now evident that low levels of $\text{H}_2\text{O}_2$, for example, can act as classical intracellular signaling molecules regulating phosphatase/kinase driven signaling pathways. The oncogenes Bcr-Abl and Flt-3 are central to the development of both chronic and acute leukemias and when these two genes are expressed on a tet regulated expression system, they are associated with increased ROS levels. The presenter demonstrated that the Flt-3 and Bcr-Abl driven ROS production ($\text{H}_2\text{O}_2$) contributes directly to genomic instability seen in tumor cells. There is also an increased flux through the PI3K/Akt survival pathway. Using siRNA and pharmacological inhibitors, it was demonstrated that the source of ROS is NADPH oxidase. Inhibition of this enzyme system lowers ROS production. Direct pharmacological inhibition of Bcr-Abl and Flt-3 is associated with marked reduction in ROS production due to ubiquitination and proteasomal destruction of p22phox which is a component of the NADPH oxidase enzyme system. This facilitates the cell death inducing properties of chemotherapeutic agents by removing the ROS element of oncogene survival signaling.

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