S.I.1.

Angiogenesis and inflammation as target for cancer prevention

Adriana Albini, Katiuscia Dallaglio, Antonino Bruno, Douglas Noonan

IRCCS "Tecnologie Avanzate e Modelli Assistenziali in Oncologia", Arcispedale S. Maria Nuova, Reggio Emilia, Italy
e-mail: adriana.albini@multimedica.it

Angiogenesis is necessary for solid tumor growth and dissemination, thus representing a promising target not only in cancer therapy but also in prevention. The principle of cancer chemoprevention is based on the use of agents that, while devoid of collateral effects, are able to interfere with processes associated with malignant progression, including cancer related inflammation (CRI). Since our solid expertise in working with many chemoprevention agents, we have observed that angiogenesis is a common and key target of most chemopreventive molecules. In this view, we termed "angioprevention" the concept that effective chemoprevention are also able to target angiogenesis . The cancer microenvironment includes a series of complex interactions and communications between tumor cells and host cells, in particular endothelial and inflammatory cells, that promote progression to malignancy. Our goal is to identify molecules and pathways to prevent tumor development by targeting the microenvironment and inflammatory angiogenesis.

We have shown that a wide array of molecules, including flavonoids, antioxidants and retinoids, act in the tumor micro-environment and inhibit the recruitment and/or activation of endothelial cells. We have shown that N-acetylcysteine (NAC), the green tea flavonoid epigallocatechin-3-gallate (EGCG) and the chalcone Xanthohumol (XN), and the Akt inhibitor deguelin all prevent angiogenesis. Interestingly, the synthetic retinoid 4-hydroxyfenretinide (4HPR) also shows similar anti-angiogenic and anti-tumor effects. We therefore demonstrated the anti angiogenic potential *in vitro* and *in vivo* of a poliphenol-rich purified extract from olive mill wastewater (OMWW-patent pending). To examine the molecular mechanisms involved in our selected compound anti angiogenic/angiopreventive activity, we have performed microarray expression profiling on endothelial cells in response to angiopreventive molecules showing their interaction with pathway involved in the inflammatory response. We have now shown that the triterpenoid CDDO-Methylesther is a remarkably potent inhibitor of angiogenesis and angiogenic tumor growth, effective at doses as low as 0.003 mg/kg body weight.

Finally, we have recently demonstrated that metformin, a wide used anti diabetic drug, represents a valid chemopreventive and angiopreventive agent, particularly in a context of obesity.

References:

Albini A, Sporn MB (2007) The tumour microenvironment as a target for chemoprevention. *Nat Rev Cancer* **7**: 139–147.

Albini A, Tosetti F, Benelli R, Noonan DM (2005) Tumor inflammatory angiogenesis and its chemoprevention. *Cancer Res* **65**: 10637–1041.

Tosetti FN, Ferrari S, De Flora, Albini A (2002) Angioprevention': angiogenesis is a common and key target for cancer chemopreventive agents. *Faseb J* **16**: 2–14.

Ferrari N, Pfeffer U, Dell'Eva R, Ambrosini C, Noonan DM, Albini A (2005) The TGF-beta family members BMP-2 and MIC-1 as mediators of the anti-angiogenic activity of 4-Hydroxyphenylretinamide. *Clin Cancer Res* **15**: 4610–4619.

Albini A, Tosetti F, Li VW, Noonan DM, Li WW (2012) Cancer prevention by targeting angiogenesis. *Nat Rev Clin Oncol* **9**: 498–509.

Dallaglio K, Bruno A, Cantelmo AR, Esposito AI, Ruggiero L, Orecchioni S, Calleri A, Bertolini F, Pfeffer U, Noonan DM, Albini A (2014) Paradoxic effects of metformin on endothelial cells and angiogenesis. *Carcinogenesis* **35**: 1055–1066.

S.I.2.

Epigenetics of dietary phytochemicals in cancer prevention: a focus on Nrf2 mediated pathway

Ah-Ng Tony Kong

Center for Cancer Prevention Research, Department of Pharmaceutics, Ernest Mario School of Pharmacy, Rutgers, the State University of New Jersey, USA
e-mail: KongT@pharmacy.rutgers.edu

Oxidative stress is caused by an imbalance of reactive oxygen species (ROS)/reactive nitrogen species (RNS) and the antioxidative stress defense systems in cells. ROS/RNS or carcinogen metabolites can attack intracellular proteins, lipids, and nucleic acids, which can result in genetic mutations, carcinogenesis, and other diseases. Nrf2 plays a critical role in the regulation of many antioxidative stress/antioxidant and detoxification enzyme genes, such as glutathione S-transferases (GSTs), NAD(P)H:quinone oxidoreductase 1 (NQO1), UDP-glucuronyl transferases (UGTs), and heme oxygenase-1 (HO-1), directly *via* the antioxidant response element (ARE). Many studies have shown that dietary phytochemicals possess cancer chemopreventive potential through the induction of Nrf2-mediated antioxidant/detoxification enzymes and anti-inflammatory signaling pathways to protect organisms against cellular damage caused by oxidative stress. In addition, carcinogenesis can be caused by epigenetic alterations including DNA methylation, histone modifications and microRNAs in tumor-suppressor genes, oncogenes or critical cellular controlling pathways. Recent studies show that many dietary phytochemicals can epigenetically modify the chromatin in many *in vitro* cell culture systems. Focusing on the Nrf2 pathway, Nrf2's CpG promoters are hypermethylated *in vitro* cell lines and *in vivo* animal tumor model and that dietary phytochemicals isothiocyanates, curcumin, tocopherols and Traditional Chinese Medicinal compounds attenuated CpG methylation and inhibited carcinogenesis. The future advancement and development of dietary phytochemicals in prevention of toxicity, carcinogenesis and other diseases requires the integration of epigenomics research

Key words: cancer chemoprevention; Nrf2, dietary phytochemicals; epigenetics; anti-oxidative stress

Acknowledgements: Supported by NIH Grants.

NOTES

S.I.3.

Targeting inflammatory pathways to prevent colorectal cancer

Chinthalapally V. Rao

Center for Cancer Prevention and Drug Development, Medical Oncology, Department of Medicine, PCS Oklahoma Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

e-mail: cv-rao@ouhsc.edu

Colorectal cancer (CRC) is fourth most common cause of cancer deaths world-wide and estimated over 1.35 million cases and about 700 000 deaths were reported annually. Importantly, recent trends show that developing countries had increased CRC incidence rates. We and others have shown that treatment with antiinflammatory phytochemicals and non-steroidal antiinflammatory drugs (NSAIDs) results in the reduction of CRC. Most regular NSAIDs block PGE_2 formation by inhibiting COX-1 and COX-2 activities. Chronic use of the most NSAIDs is associated with unfavorable gastrointestinal side effects. While agents that target specifically COX-2 are noteworthy because of their superior clinical efficacy in inflammatory disorders and cancer prevention, they also contribute to cardiovascular (CV) risk. Recently we have designed various strategies to overcome CV risk without limiting the colon tumor inhibitory potential. In this context, we have established several modified NSAIDs such as nitric-oxide (NO) releasing NSAIDs, phospho-NSAIDs, licofelone to mention only few. We will discuss the utility of several of these agents in the inhibition of colon tumors development in rodent models. In addition, we will present information on the inflammation that directly alters tumor immune function that in turn modulates the colon tumor growth and its metastasis.

Key words: colorectal cancer; inflammation; chemoprevention; NSAIDs; cardiovascular risk

NOTES

S.I.4.

Isothiocyanates potentiate anti-proliferative and anti-metastatic activity of lapatinib in HER2-positive breast cancer cells

Angelika Kaczyńska, Anna Herman-Antosiewicz

Department of Molecular Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdańsk, Poland
e-mail: angelikakaczynska.biotech@gmail.com

Human epidermal growth factor receptor 2 (HER2) is overproduced in nearly 20–25% of all breast cancer cases and it correlates with poor clinical outcomes. Intracellular signal transduction cascades initiated from amplified receptors, such as Akt-mTOR-S6K, leads to uncontrolled proliferation, evasion of apoptosis, neoangiogenesis and enhances cell motility, increasing metastatic capacity.

Lapatinib is a small-molecule drug that inhibits signaling from HER2 by blocking ATP-binding site in cytoplasmatic domain of this receptor. However, in spite of great success in breast cancer therapy, primary or acquired resistance to lapatinib still occurs, even when it is used in combination with other commercially available anti-HER2 agents (e.g. trastuzumab). Additionally, prevalence of serious side effects, become a major limitation of such therapies.

We hypothesized that combined treatment, targeting different levels of signal transduction pathway from HER2, may have stronger cytotoxic effect on breast cancer cells than therapy targeting the receptor only. Innovativeness of the proposed approach relies on combination of commercially available drug, lapatinib, with one of plant-derived isothiocyanates: sulforaphane, erucin or sulforaphene, as it has been shown previously that sulforaphane inhibits Akt-mTOR-S6K1 pathway in breast cancer cells. Using two HER2 overexpressing breast cancer cell lines, SKBR-3 and BT-474, we demonstrate that each combination (lapatinib with sulforaphane, erucin or sulforaphene) considerably decreases phosphorylation of proteins crucial in signal transduction from HER2, cell viability and migration potential as well as more efficiently induces apoptosis as compared to activity of each of these agents alone. Lapatinib with erucin is the most effective combination.

It is noteworthy that long-term exposure to lapatinib causes elimination of drug-sensitive cells and at the same time increases probability of selection of lapatinib-resistant cells whose percentage rises in the cell population with time and may lead to metastasis. In our work we also made an *in vitro* simulation of the drug-dependent selection of resistant cells during lapatinib therapy and tested efficiency of combined treatment. Lapatinib-sensitive (SKBR-3) and lapatinib-resistant cells (SKBR-3-lapR) were mixed in different proportions to simulate resistance acquisition process. Our model shows, that combination of lapatinib with any of isothiocyanates causes significant decline of cell viability, phosphorylation of Akt kinase and ribosomal S6 protein (a substrate for S6K) as well as cell motility as compared to activity of each agent alone, even when percentage of resistant cells increases, and drug sensitivity decreases. However, the most effective is combination of lapatinib with erucin.

These findings might support the optimization of therapy based on lapatinib treatment.

Key words: lapatinib; isothiocyanates; breast cancer; HER2 receptor

Acknowlegments: This work was supported by National Science Center (Poland), project no. N N301 601740.

NOTES