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L'Aquila, 11-14 June 2018

Rettorato GSSI Gran Sasso Science Institute
Via Michele Iacobucci, 2 - 67100 L'Aquila

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metastasis promoting "Warburg" effect in TrkAIII expressing tumour cells.

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2. Schramm A et al., Br J Cancer 2012, 107:1409-17.
3. Farina AR et al., Oncotarget 2015, 6:35636-51.
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EFFECTS OF NATURAL COMPOUNDS ON THE OXIDATIVE BALANCE IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Most of the recently developed anticancer drugs induce apoptotic cell death in tumor cells through up-regulating the intracellular ROS levels. New evidence suggests the promising role of curcumin, a yellow-gold color phytochemical turmeric, isolated from root of the *Curcuma longa*, and of graviola, (*Annona muricata*), a tropical plant belonging to family Annonaceae, known for its medicinal uses, in the treatment of cancer[1-2]. In our study we analyzed the effects on proliferation and apoptosis in ALL and Jurkat cell line of graviola and curcumin, alone and in combination with various chemotherapeutic agents (Daunorubicin, L-Asparaginase, Metotrexate, Vincristine and Desametazone). The proliferation, apoptosis, cell cycle and ROS production, before and after treatment with a ROS inhibitor, were investigated. Cell fragmentation was observed in Time lapse Imaging.
Results: Our preliminary data showed an inhibition of proliferation and an apoptosis induction after 20µg/mL both of curcumin and graviola treatment for 24h.
The combined treatment of curcumin respectively with Daunorubicin, L-ASPA, Vincristine and Desametazone showed a significant shift from early to late apoptosis after 24h, using the lowest effective concentration of drugs, compared to the higher dose of drugs alone: the average apoptotic increase was 49 ± 6.3% ($p<0.05$). Confocal analysis confirmed the internalization of curcumin in Jurkat cells, leading to cytoplasmic and partly nuclear fragmentation, especially when combined with vincristine. Curcumin treatment increased intracellular ROS levels, thus inducing apoptosis in leukemia cells. This selective activity could be attributed to the different redox states between healthy cells and leukemic cells. Curcumin has been described as an inducer of apoptosis and cell cycle arrest *via* regulating multiple cancer signaling pathways. The molecular insight onto curcumin-mediated anticancer property in leukemia suppression remains to be elucidated.

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2. Deep G et al. Sci Rep 2016,6:23135

H_2O_2 INDUCES NECROPTOSIS IN MESOANGIOBLAST STEM CELLS

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Stem cells are used in regenerative medicine, but their therapeutic efficacy is compromised by their huge death during the first days post-transplantation. Indeed, the microenvironment within damaged tissues is hostile for stem cell survival mainly due to oxidative stress. H_2O_2 may play a relevant role in inducing death of the injected cells. The aim of our study was to determine the mechanism of mesoangioblast (A6) cell death after an H_2O_2 treatment.
FACS analysis with annV/PI showed that H_2O_2 induced a dose and time-dependent decrement in A6 viability. We have also found an increase in caspases 8, 9 and 3 activity after the treatment. To assess their involvement in cell death, the pan caspase inhibitor Z-VAD was used. Neither early apoptosis, nor late apoptosis/necrosis, nor necrosis were reduced, suggesting that the cell death induced by H_2O_2 was caspase-independent. Then, we tested whether H_2O_2 is responsible for the autophagy activation. To study autophagy we evaluated the expression of specific markers. H_2O_2 decreased beclin1, Atg5, Atg7 and the ratio LC3II/I, in a dose dependent way. At the same time it increased p62 protein expression indicating an impaired autophagic flux, also confirmed by the increase of pAKT, responsible for the activation of mTOR, a negative regulator of autophagy. According to these data A6 treatment with H_2O_2 seems to not induce nor apoptosis or autophagy. For this reason we hypothesized the activation of necroptosis, a specific form of caspase-independent, non-apoptotic or necrotic cell death. To confirm whether the observed cell death was due to enhanced necroptosis, the proportion of necrotic cells was determined by annV/PI staining. FACS analysis showed an increase in percentage of both late apoptotic/necrotic and necrotic cells, which were further increased by pretreatment with Z-VAD. To investigate the relationship between physiological autophagy and necroptosis, cells were treated with H_2O_2 in the presence of the autophagic inhibitor 3MA. AnnV/PI staining showed that the inhibition of autophagy by 3MA significantly enhanced necroptosis in A6 treated cells. Conversely, 3MA had no effect on apoptosis. In conclusion, our data indicate that the cytotoxicity of H_2O_2 in A6 mainly occurred *via* the induction of necroptosis, enhanced by both apoptosis and autophagy inhibition.

DO AGING-MEDIATED EPIGENETIC CHANGES CAUSE METABOLIC REMODELLING DURING AGING?

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The activity of the heart is highly dependent upon metabolism due to its high and constant energy requirements. To meet its energy needs, the heart is a metabolically dynamic organ able to use different substrates (e.g., fatty acid, glucose lactate and ketone bodies) as energy sources. This property allows the heart to choose the most efficient substrate for each physiological condition[1]. In normal heart, 95% of energy is obtained from mitochondrial oxidative phosphorylation, of which 50%-75% is fatty acid oxidation and the remaining (25%-50%) is glucose oxidation. During aging, similarly to what happens in heart failure, the heart loses this dynamicity and shifts from mitochondrial oxidation to glycolysis[2]. This process, known as metabolic remodelling, causes an "energy deficit" that contributes to impairment of cardiac function in the elderly[3]. The molecular mechanisms triggering this remodelling are not completely understood.
Histone marks, such as acetylation and methylation of histone H3, have an important role in defining the transcription program at the base of cardiomyocyte differentiation and heart homeostasis in the adult[4]. Our preliminary data support the hypothesis that changes in the genomic distribution of histone marks have a role in defining the transcription changes of genes encoding enzymes