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**Spermine metabolite, hydrogen peroxide and aldehyde, induce the apoptosis of neuroblastoma cells associated with an increase in p53, Caspase-3 and miR-34a**Enzo Agostinelli<sup>1,2</sup>, Yuta Kanamori<sup>1</sup>, Alessia Finotti<sup>3</sup>, Laura Di Magno<sup>4</sup>, Gianluca Canetti<sup>5</sup>, Shinji Ohkubo<sup>6</sup>, Fabio Timeus<sup>7</sup>, Jessica Gasparello<sup>8</sup>, Italia di Liegro<sup>9</sup>, Roberto Gamburzi<sup>10</sup><sup>1</sup>Department of Biochemical Sciences, University of Rome "La Sapienza", Rome; <sup>2</sup>International Polyamines Foundation ONLUS, Rome; <sup>3</sup>Department of Life Sciences and Biotechnology, Biochemistry and Molecular Biology Section, University of Ferrara, Ferrara; <sup>4</sup>Department of Molecular Medicine, University of Rome "La Sapienza", Rome; <sup>5</sup>Paediatric Onco-haematology, Regina Margherita Children's Hospital, Turin; <sup>6</sup>Department of Biomedicine, Neurosciences and Advanced Diagnostics (Bi.N.D.), University of Palermo, Palermo, Italy  
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Neuroblastoma (NB) is a common malignant solid tumour in children, which originates from the sympathoadrenal lineage of neural crest and accounts for 15% of childhood cancer mortality. Amplification of the oncogene N-Myc is a well-established poor prognostic marker for neuroblastoma. Whilst N-Myc amplification status strongly correlates with higher tumour aggression and resistance to treatment. Therefore, new therapies for patients with N-Myc amplified NB need to be developed. The *in situ* formation of cytotoxic polyamine metabolites by bovine serum amino oxidase (BSAO) is a recent approach in cancer enzymotherapy. It has been demonstrated that BSAO and spermine (SPM) addition to cancer cells induces cell growth inhibition and apoptosis through the oxidative stress caused by polyamine metabolites, H<sub>2</sub>O<sub>2</sub> and aldehydes, produced by the oxidative reaction (1). The cytotoxic effect induced by BSAO and SPM was evaluated by both clonogenic and MTT assays. The detection of apoptosis of the NB cells was evaluated by flow cytometry after Annexin V-FITC labelling and DNA staining with propidium iodide. The percentages of Annexin V-positive cells matched quite well with those of cells exhibiting a hypodiploid sub-G1 peak. An increase in mitochondrial membrane depolarization (MMD) was found in the NB cells treated with the enzymatic system. The mitochondrial membrane potential activity was examined by flow cytometric assays, labelling cells with the probe JC1 dye. We also analysed, by RT-qPCR, the transcript of some genes involved in the apoptotic process, to determine the possible down- or upregulation of mRNAs following treatment of the SJ-N-KP and the N-Myc-amplified IMR-5 cell lines with BSAO and SPM. The experiments were carried out considering the pro-apoptotic genes p53, PUMA and Caspase-3. Following treatment with BSAO and SPM, both cell lines displayed increased mRNA levels for all the pro-apoptotic genes. Notably, the level of the pro-apoptotic Sirt1 inhibitor microRNA, miR-34a, significantly increased in the SJ-N-KP and IMR5 cells treated with BSAO and SPM. Western blot analysis with PARP and Caspase-3 antibody supported the concept that BSAO/SPM treatment induced high levels of apoptosis in the NB cell lines. The major conclusion is that BSAO/SPM treatment leads to the anti-proliferative and cytotoxic activity of both NB cell lines, associated with the activation of apoptosis. Moreover, these findings suggest that enzymatic spermine metabolite may be a powerful tool for the development of novel anticancer treatments.

(1) Amendola et al. Reactive oxygen species spermine metabolites generated from amine oxidases and radiation represent a therapeutic gain in cancer treatments (2013) International Journal of Oncology, 43 (3), pp. 813-820.

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**Perspectives of taurine derivatives**

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Active halogen taurine derivatives are basically the naturally occurring N-chlorotaurine (NCT) and N-bromotaurine (NBrT), which are produced by activated human neutrophilic and eosinophilic granulocytes and monocytes. Due to their oxidative mechanisms of action, they exhibit broad spectrum microbicidal activity against bacteria, fungi, viruses and protozoa, as well as anti-inflammatory properties, such as the downregulation of pro-inflammatory factors and cytokines such as NF- $\kappa$ B, interleukins, prostaglandin E2, tumor necrosis factor, neopterin and others. In particular, NCT has been developed as a natural anti-infective and antiseptic for topical application at different body sites, particularly sensitive ones, for instance, the eye, ear, ulcerated skin and the urinary bladder. NBrT was applied successfully on acne. Since both natural compounds have to be cooled for longer storage, which is only sufficient for NCT, synthetic active halogen compounds have been created that possess a higher stability. In particular, bromamine T (BAT) is a compound which has attracted much interest since it resembles the properties of NBrT. Future perspectives for application are the following: NCT is particularly suited for purulent infections of sensitive body sites as mentioned above. The reasons are its mild activity, low chlorine consumption and the enhancement of microbicidal activity in body fluids and exudates by transhalogenation. The inhalation of NCT in chronic bronchitis or cystic fibrosis is a very promising investigative field. Bromamines appear to be suited for treatment of infections, as well, as demonstrated on the skin for acne and herpes zoster. In addition, they exert multiple anti-proliferative effects against tumor cells. Thus, oncology has become a topic of great interest, particularly as regards BAT and NBrT.

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**IL-12 regulates the expansion of effector-like NK cells induced by IL-15/18 and alters their phenotypes and functions**Naoto Oka<sup>1</sup>, Tzvetanka Markova<sup>2</sup>, Magdalena Pencheva<sup>2</sup>, Wen Li<sup>3</sup>, Kyousuke Yamanishi<sup>4</sup>, Hiromichi Yamanishi<sup>4</sup>, Haruki Okamura<sup>3</sup><sup>1</sup>Department of Otorhinolaryngology-Head and Neck Surgery, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; <sup>2</sup>Department of Pharmacology, Medical University, Sofia, Bulgaria; <sup>3</sup>Department of Psycho-immunology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; <sup>4</sup>Hirakata General Hospital for Developmental Disorders Hirakata, Osaka, Japan  
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Recent progress in cancer immunotherapy has been greatly encouraging; however, the limited efficacy prompts researchers to improve therapy. The exhaustion of effector lymphocytes may bring about the tumor evasion of immune attack. NK cells are critical for cancer immunotherapy, as they are not homogeneous in phenotype and function. Recent studies have demonstrated that the stimulation of peripheral NK cells by IL-12/15/18 generates longer-living, memory-like NK cells. In the present study, we demonstrated that this process could be separated into phases of effector-like cells induced by IL-15/18 and longer-living cells differentiated by IL-12. Freshly prepared splenic NK cells expressed IL-15Rs and IL-18Rs, and rapidly began to proliferate by the stimuli of combination of IL-15 and IL-18 (IL-15/18). These proliferating cells highly expressed various activation markers and exerted potent cytotoxic effects. They expressed IL-12 receptors,  $\beta$ 1 and  $\beta$ 2; however, they did not secrete cytokines, while they had a high potential to produce IFN- $\gamma$  in response to IL-12. IL-12 strongly activated STAT4 in the cells activated by IL-15/18, upregulated p21 and p27, and led to withdrawal from the cell cycle suppressing cell expansion. In parallel, IL-12 rapidly induced IFN- $\gamma$  production, greatly altered the expression of surface molecules, reduced cytotoxicity, and generated longer-living cells. Notably, a large proportion of IL-15/18-induced cells strongly expressed PD-1 together with activation molecules, whereas NK cells induced by IL-15/18 and IL-12 expressed high levels of TIM-3, LAG-3 and NKG2A. Furthermore, the latter spontaneously secreted IL-10 and TGF- $\beta$  during prolonged incubation. These results indicated that IL-12 regulated the expansion of IL-15/18-induced, effector-like NK cells, generating longer-living cells. In addition, peripheral NK cells were suggested to be differentially regulated by various ligands of immune checkpoint molecules at different stages of differentiation. IL-12 signaling may be involved in the terminal differentiation of NK cells and influences the population size of effector NK cells. These findings may also give suggestion to understanding of mechanism of exhaustion of NK cells.

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**Membrane protein inventory of human pheochromocytoma and paraganglioma**Ondrej Vit<sup>1</sup>, Karel Pacak<sup>2</sup>, Jiri Petrak<sup>1</sup><sup>1</sup>BIOCEV, First Faculty of Medicine, Charles University in Prague, Czech Republic; <sup>2</sup>National Institutes of Child Health and Human Development (NICHD), Bethesda, Maryland, USA  
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Pheochromocytoma and paraganglioma (PHEO/PGL) are rare neuroendocrine tumors derived from adrenal medulla or extradrenal paraganglia, respectively. The prognosis of patients with malignant PHEO/PGL is poor, and specific molecular targets for novel therapies are therefore required. Integral membrane proteins (IMPs) expressed by tumors represent such potential therapeutic targets, due to their specific functions and localization. Our goal is to provide a detailed inventory of membrane proteome of human PHEO and PGL that could help identify novel drug targets and diagnostic markers. IMPs are coded by roughly 25% of human genes; however, our knowledge of the IMP repertoire expressed by specific tumors is limited. Their amphipathic nature, the lack of trypsin cleavage sites and their relatively low expression hinder the proteomics analysis of IMPs. The specific physicochemical properties of IMPs require specific analytical strategies. In order to maximize the coverage of the PHEO/PGL membrane proteome, we combined a standard trypsin-based approach with a selective isolation of (extramembrane) glycopeptides and with our recently introduced hPTC method, which selectively targets (transmembrane) hydrophobic segments of IMPs, into a multi-pronged 'Pitchfork' strategy. The methods included in the Pitchfork strategy target different features of IMPs, are complementary, and allow for the identification of a significant portion of the membrane proteome expressed by PHEO/PGL. On average, we identified 900-1300 IMPs in each PHEO/PGL tumor sample analyzed to date. Our current dataset represents nearly 2,200 unique IMPs identified in PHEO and a similar number in PGL tumor samples. Among the identified proteins, we observed several proteins expressed in tumor tissue, but not in healthy adrenal medulla. Such proteins are currently studied in detail as potential drug targets or disease markers.

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