Conclusions: Modulation of the cellular NO concentration causes extracellular singlet oxygen generation and inactivation of tumor cell protective catalase.

http://dx.doi.org/10.1016/j.redox.2015.09.018

Young Investigation Session Selected Oral Communications

Detection Of Human Papilloma Virus (Type 16 And 18) In Cytological Samples In Patients With Lung Cancer. An Evaluation By Chromogenic In Situ Hybridization Technique

Nada Al-Shabbani

Al Karama Teaching Hospital, Baghdad, Iraq

Background: Lung cancer is a common neoplasm. Some studies suggested that HPV may play an etiologic role in bronchial carcinogenesis and possibility of a latent HPV infection as a cocarcinogen cannot be excluded. HPV (high risk types) 16 &18 may affect the cell cycle and inhibit apoptosis, allowing uncontrolled cell division.
Aims of study: 1. To use bronchial wash as a non invasive procedure to determine the presence of DNA of high risk types (16 &18)of HPV in patients with lung cancer using in situ hybridization technique. 2. To Find the association of the score or intensity of viral in situ hybridization signals with histopathological types.
Patients, Materials and methods: A prospective study, whereby bronchial wash of 50 patients diagnosed cytologicaly as having lung cancer together with samples from 30 patient having chronic illness as control.
Results: The most affected age group was 66–70 years (28% of the cases). Males were affected more frequently than females (64.0%). Regarding HPV16 & 18 and histological types of lung cancer the commonest effected type was squamouse cell carcinoma showed positive signals in 32% and 28% for HPV16&HPV18 respectively. Percentage score and intensity score: Lung cancer samples showed low intensity signals for human papilloma virus16 in 63.6% of samples and 44.5% for human papilloma virus 18.
Conclusions: Human papilloma virus 16 and 18 was detected in cytological bronchial samples of patients with lung cancer in relatively high percentage

http://dx.doi.org/10.1016/j.redox.2015.09.019

Stabilization Of Apoptotic Cells: Generation Of Zombie Cells

José A. Sánchez Alcázar, Manuel Oropesa Ávila, Yuniesky Andrade Talavera, Juan Garrido Maraver, Isabel de Lavera, Mario de la Mata, David Cotán, Marina Villanueva Paz, Ana Delgado Pavón, Elisabet Alcocer Gómez, Antonio Rodríguez Moreno

Universidad Pablo de Olavide, Sevilla, Spain

Apoptosis is characterized by degradation of cell components but plasma membrane remains intact. Apoptotic microtubule network (AMN) is organized during apoptosis forming a cortical structure beneath plasma membrane that maintains plasma membrane integrity. Apoptotic cells are also characterized by high reactive oxygen species (ROS) production that can be potentially harmful for the cell. The aim of this study was to develop a method that allows stabilizing apoptotic cells for diagnostic and therapeutic applications. We were able by using a cocktail composed of taxol (a microtubule stabilizer), Zn^{2+} (a caspase inhibitor) and coenzyme Q_{10} (a lipid antioxidant) to stabilize H460 apoptotic cells in cell cultures for at least 72 hours preventing secondary necrosis. Stabilized apoptotic cells maintain many apoptotic cells characteristics such as the presence of apoptotic microtubules, plasma membrane integrity, low intracellular calcium levels, plasma membrane potential, PS externalization and ability of being phagocytosed.

Stabilized apoptotic cells can be considered as dying cells in which the cellular cortex and plasma membrane are maintained intact or alive. In a metaphorical sense, we can consider them as “living dead” or “zombie cells”.

Stabilization of apoptotic cells can be used for reliable detection and quantification of apoptosis in cultured cells and may allow a safer administration of apoptotic cells in clinical applications. Furthermore, it opens new avenues in the functional reconstruction of apoptotic cells for longer preservation.

http://dx.doi.org/10.1016/j.redox.2015.09.020

Session 3: Nitric Oxide: Proliferation and Epithelial-Mesenchymal Transition Moderator: Dr. Timothy R. Billiar INVITED SPEAKERS

STAT3 Regulation By S-Nitrosylation: Implication In Cancer

Inderjit Singh, Jinsu Kim, Avtar K. Singh, Anand K. Sharma, Je-Seong Won

Departments of Pediatrics, Pathology and Laboratory Medicine and Radiation Oncology. Medical University of South Carolina, Charleston, SC, USA

In this study, we assessed S-nitrosylation-based regulation of Janus-activated kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway. Our studies show that STAT3 in stimulated microglia underwent two distinct redox-dependent modifications, S-nitrosylation and S-glutathionylation. STAT3 S-nitrosylation was associated with inducible nitric oxide synthase (iNOS)-produced nitric oxide (NO) and S-nitrosoglutathione (GSNO), whereas S-glutathionylation of STAT3 was associated with cellular oxidative stress. NO produced by iNOS or treatment of microglia with exogenous GSNO inhibited STAT3 activation via inhibiting STAT3 phosphorylation (Tyr705). Consequently, the interleukin-6 (IL-6)-induced microglial proliferation and associated gene expressions were also reduced. In cell-free kinase assay using purified JAK2 and STAT3, STAT3 phosphorylation was inhibited by its selective preincubation with GSNO, but not by preincubation of JAK2 with GSNO, indicating that GSNO-mediated mechanisms inhibit STAT3 phosphorylation through S-nitrosylation of STAT3 rather than JAK2. In this study, we identified that Cys259 was the target Cys residue of GSNO-mediated S-nitrosylation of STAT3. The replacement of Cys259 residue with Ala abolished the inhibitory role of GSNO in IL-6-induced STAT3 phosphorylation and transactivation, suggesting the role of Cys259S-nitrosylation in STAT3phosphorylation.

Since STAT3 activation is involved in tumor progression and metastasis, we investigated the effect of GSNO in cell culture and mouse xenograft model of head and neck squamous cell carcinoma (HNSCC). GSNO treatment of HNSCCN cell lines reversibly decreases the activation (phosphorylation) of STAT3 in a concentration dependent manner. The reduced STAT3/NF-kB activity by GSNO correlated with decreased cell proliferation and increased apoptosis of HNSCC cells. In HNSCC mouse xenograft model, the tumor growth was reduced by systemic treatment with GSNO and was further reduced when the treatment combined with radiation and cisplatin. Accordingly, GSNO treatment also resulted in decreased levels of pSTAT3 and tumor growth regulators (ie. cyclin D2, VEGF and Bcl-2) in tumor tissue. In summary, these findings have implications for the development of new therapeutics targeting of STAT3 for treating diseases associated with inflammatory/immune responses and abnormal cell proliferation, including cancer.

http://dx.doi.org/10.1016/j.redox.2015.09.021

Mechanisms Of Hypoxia-Induced Immune Escape In Cancer And Their Regulation By Nitric Oxide

Charles Graham, Ivraym Barsoum, Judy Kim, Madison Black, Robert D. Siemens

Queen's University, Kingston, Canada

The acquired ability of tumour cells to avoid destruction by immune effector mechanisms (immune escape) is important for malignant progression. Also associated with malignant progression is tumour hypoxia, which induces aggressive phenotypes such as invasion, metastasis and drug resistance in cancer cells. Our studies revealed that hypoxia contributes to escape from innate immunity by increasing tumour cell expression of the metalloproteinase ADAM10 in a manner dependent on accumulation of the alpha subunit of the transcription factor hypoxia-inducible factor-1 (HIF-1α). Increased ADAM10 expression leads to shedding of the NK cell-activating ligand, MICA, from the surface of tumour cells, thereby resulting in resistance to NK cell-mediated lysis. Our more recent studies demonstrated that hypoxia, also via HIF-1α accumulation, increases the expression of the inhibitory co-stimulatory ligand PD-L1 on tumour cells. Elevated PD-L1 expression leads to escape from adaptive immunity via increased apoptosis of $CD8^+$ cytotoxic T lymphocytes. Accumulating evidence indicates that hypoxia-induced acquisition of malignant phenotypes, including immune escape, is in part due to impaired nitric oxide (NO)-mediated activation of cGMP signalling and that restoration of cGMP signalling prevents such hypoxic responses. We have shown that NO/cGMP signalling inhibits hypoxia-induced malignant phenotypes likely in part by interfering with HIF-1α accumulation via a mechanism involving calpain. These findings indicate that activation of NO/cGMP signalling may have useful applications in cancer therapy.

http://dx.doi.org/10.1016/j.redox.2015.09.022

Evaluating The Role Of Nitric Oxide Synthase In Oncogenic Ras-Driven Tumorigenesis

Chris Counter

Duke University Medical Center, Durham, USA

We previously reported that oncogenic KRAS activation of the PI3K/AKT pathway stimulates the remaining wild-type HRAS and NRAS proteins in a manner dependent upon both eNOS expression and C118 in HRAS and NRAS, which promoted tumor growth. Interestingly however, we recently found that loss of wild-type HRAS, NRAS, and even more potently, loss of both of these genes actually enhanced oncogenic KRAS-driven early tumorigenesis. Taken together, these results indicate that wild-type RAS proteins are tumor suppressing early in tumorigenesis, but tumor promoting in more malignant settings. Knock-in of a C118S mutation into an endogenous wild-type *RAS* gene did not, however, hamper oncogenic KRAS-driven tumor initiation. As such, redox-dependent reactions with C118 of wild-type RAS proteins are unlikely to be responsible for the tumor suppressive role of wild-type RAS proteins. This suggests that the redox-dependent reactions with C118 of wild-type RAS proteins are more important in more malignant settings. Given this, it stands to reason that inhibiting redox-dependent reactions like *S*-nitrosylation of wild-type RAS proteins may be more effective in established cancer settings. Indeed, we find that in three different models of KRAS-driven cancers-skin, pancreatic and lung- the general NOS inhibitor L-NAME reduced tumor burden and/or extended the lifespan of mice. Since oncogenic RAS has so far proven refractory to pharmacologic inhibition, targeting NOS activity may be an actionable approach to inhibiting RAS signaling for the treatment of a broad spectrum of cancers.

http://dx.doi.org/10.1016/j.redox.2015.09.023

Young Investigation Session Selected Oral Communications

Nitric Oxide And Hypoxia Response In Pluripotent Stem Cells

Estefanía Caballano Infantes, Ana Belén Hitos Prados, Irene Díaz Contreras, Gladys M. Cahuana, Abdelkrim Hmadcha, Franz Martín Bermudo, Bernat Soria, Juan R. Tejedo Huamán, Francisco J. Bedoya Bergua

Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Sevilla, Spain

The expansion of pluripotent cells (ESCs and iPSCs) under conditions that maintain their pluripotency is necessary to implement a cell therapy program. Previously, we have described that low nitric oxide (NO) donor diethylenetriamine/nitric oxide adduct (DETA-NO) added to the culture medium, promote the expansion of these cell types. The molecular mechanisms are not yet known. We present evidences that ESC and iPSCs in normoxia in presence of low NO triggers a similar response to hypoxia, thus maintaining the pluripotency. We have studied the stability of HIF-1α (Hypoxia Inducible Factor) in presence of low NO. Because of the close relationship between hypoxia, metabolism, mitochondrial function and pluripotency we have analyzed by q RT-PCR the expression of genes involved in the glucose metabolism such as: HK2, LDHA and PDK1; besides other HIF-1α target gene. We further analyzed the expression of genes involved in mitochondrial biogenesis such as PGC1α, TFAM and NRF1 and we have