

observed that low NO maintains the same pattern of expression that in hypoxia. The study of the mitochondrial membrane potential using Mito-Tracker dye showed that NO decrease the mitochondrial function. We will analyze other metabolic parameters, to determinate if low NO regulates mitochondrial function and mimics Hypoxia Response. The knowledge of the role of NO in the Hypoxia Response and the mechanism that helps to maintain self-renewal in pluripotent cells in normoxia, can help to the design of culture media where NO could be optimal for stem cell expansion in the performance of future cell therapies.

<http://dx.doi.org/10.1016/j.redox.2015.09.024>

Redox Regulation Of Metabolic And Signaling Pathways By Thioredoxin And Glutaredoxin In Nitric Oxide Treated Hepatoblastoma Cells

C. Alicia Padilla Peña, Raúl González, María J. López-Grueso, J. Antonio Bárcena

Department of Biochemistry and Molecular Biology, IMIBIC, University of Córdoba, Córdoba, Spain

Background: NO has an antiproliferative action on HepG2 cells and Thioredoxin (Trx) and Glutaredoxin (Grx) have denitrosilase and deglutathionylase activities.

Aims: To ascertain whether Trx and/or Grx systems intermediate the anti-proliferative effect of NO on hepatoblastoma cells by modulating the redox-state of key proteins.

Methods: HepG2 cells overexpressing Nitric Oxide Synthase-3 (NOS-3) were transfected with specific siRNA to silence Trx1 and Grx1. The expression and thiolic redox state of proteins were determined by Western blot and redox mobility shift assay.

Results: Overexpression of NOS3 increased the levels and activities of proteins of the redoxin systems, Trx1, Grx1, TrxR1 and TxnIP, and the levels of signaling proteins (Akt1, pAkt1^{Ser473}, MapK, pMapK, Stat3, Fas). The thiolic redox state of Trx1, Grx1 and Akt1 shifted to more oxidized. Increases were also observed in Pro-apoptotic Caspase-3 fragment levels; caspase 3, 8 and 9 activities; antiapoptotic (Bcl-2); mitochondrial energetic (Aco2) and heme (Urod) metabolism; Glycolysis (Pkm2); and pentose phosphate pathway (Tkt). However, two cytosolic proteins related to iron (Aco1) and one carbon (Mat2) metabolism decreased markedly. Moreover, the redox state of Urod and Aco1 shifted to more oxidized cysteines.

Trx1 or Grx1 silencing augmented Tyr nitration and diminished cell proliferation in WT cells, but attenuated the antiproliferative effect on NO, the increase of Fas, Akt1 and pAkt1^{Ser473} and the oxidative modification of Akt1 in NOS3 cells.

Conclusions: Trx1 and Grx1 exert contradictory influences on HepG2 cells. They are required for proliferation but they also contribute to antiproliferative effect of NO, associated to Akt1 redox changes.

<http://dx.doi.org/10.1016/j.redox.2015.09.025>

Session 4: Regulation of Immune Response by Nitric Oxide

Moderator: Dr. Khosrow Kashfi
INVITED SPEAKERS

Cellular Protective Mechanisms Of Inducible Nitric Oxide Synthase

Timothy R. Billiar

Department of Surgery, University of Pittsburgh, USA

The inducible nitric oxide synthase (iNOS) is expressed constitutively but also induced in a number of epithelial cell types. iNOS regulates a number of cellular processes in these cell types without exerting toxicity. Among these functions is protection from cellular injury mediated by pro-apoptotic signals. We have had long-standing interest in the cell protective roles of iNOS in hepatocytes. We demonstrated that the upregulation of iNOS protects hepatocytes and the liver from TNF-mediated toxicity. This includes the inhibition of caspase activity through s-nitrosation. However, some of the effects are mediated through cGMP. Exploration into the mechanisms of the cGMP-mediated protection identified a role for the iNOS/NO/cGMP pathway in the activation of ADAM17 (TACE), which is a sheddase that cleaves a number of cell surface receptors including TNF receptor type 1 (TNFR1). The activation is associated with the phosphorylation of TACE.

The iNOS/NO/cGMP/TACE pathway can be augmented by PDE5 inhibitors and reduce organ injury in the setting of sepsis. The implications go beyond acute pathophysiology and may be important to the mechanisms of iNOS in promoting aggressive cancers.

<http://dx.doi.org/10.1016/j.redox.2015.09.026>

Post-Translational Nitric Oxide-Dependent Modifications In Immune System

Antonio Martínez-Ruiz

Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa (IP) Madrid, Spain

Nitric oxide non classical signalling is exerted through a series of covalent protein post-translational modifications, which include modification of cysteine residues by S-nitrosylation and S-glutathionylation.

A key process in adaptive immunity is the immune synapse that tightly couples T cells with antigen presenting cells, triggering antigen recognition by T cells. In this highly regulated process, we have shown that eNOS is activated, inducing protein S-nitrosylation. While both N-Ras and K-Ras are present in T cells, only N-Ras, which colocalizes in the Golgi with eNOS, is S-nitrosylated and activated during the immune synapse, providing an example of short-range selectivity of NO signalling through S-nitrosylation.

We have developed proteomic methods to detect S-nitrosylation and reversible cysteine oxidations. We have applied them to detecting S-nitrosylated proteins in macrophage activation, highlighting the role of denitrosylase mechanism, particularly the thioredoxin pathway, in protecting macrophages from self-modification.

We have also applied these proteomic methods to studying protein modification in acute hypoxia. In endothelial cells, there is an increase in cysteine oxidation in several proteins that can mediate acute responses to hypoxia prior to the activation of the HIF pathway, and we are currently studying in more detail the role of protein S-nitrosylation.

We have also recently shown that acute hypoxia produces a superoxide burst in cells, which can be converted in an oxidative signal through protein cysteine modification, and we are unraveling the molecular mechanisms producing this superoxide burst in mitochondria.

<http://dx.doi.org/10.1016/j.redox.2015.09.027>

Macrophage Polarization In The Tumor Microenvironment

Bernhard Brüne, Andreas Weigert, Nathalie Dehne

Institute of Biochemistry I, Faculty of Medicine, Goethe-University Frankfurt, Frankfurt, Germany

Background: Tumor associated macrophages (TAMs) are known to support tumor progression and their accumulation is generally associated with poor prognosis. The shift from a tumor-attacking to a tumor-supportive macrophage phenotype is based on an educational program that, at least in part, is initiated by apoptotic tumor cells.

Aims: We explored the macrophage phenotype shift during tumor progression by analyzing the macrophage NO-output system and examining potential NO targets.

Methods: Biochemical and Molecular Biology-orientated cell culture experiments, in part using 3D-tumor spheroid models as well as animal experiments were used.

Results: Apoptotic cells polarize macrophages towards a healing, tumor-supportive phenotype. Soluble mediators released from apoptotic cells, among them the lipid sphingosine-1-phosphate (S1P), cause expression of arginase 2 in macrophages, thereby lowering citrulline/NO formation but enhancing ornithine production. Mechanistically, this is achieved via the S1P2 receptor and the CRE (cAMP-response element) binding site in the arginase 2 promoter. Reduced NO-formation is also seen in ex vivo macrophages from a xenograft model allowing restricted vs. unrestricted tumor growth based on tumor-associated S1P-formation. The theoretical ability of NO to target hypoxia-inducible factor-1 (HIF-1) and jumonji histone demethylases (JHDMs) in cells of the tumor microenvironment will be discussed in light of the iNOS/arginase balance. Moreover, data on the importance of HIF-1 in macrophages for their interaction with tumor cells, polarization, and angiogenic potential will be presented.

Conclusions: We hypothesize that apoptotic death of tumor cells and associated macrophage activation facilitates the progression of malignant disease. The macrophage polarization program affects the NO-output system and the capacity of macrophages to support or restrict tumor growth.

<http://dx.doi.org/10.1016/j.redox.2015.09.028>

Young Investigation Session Selected Oral Communications

Polymorphisms In The Nitric-Oxide Synthase 2 Gene And Prostate Cancer Pathogenesis

Charlotta Ryk, Petra de Verdier, Emmie Montgomery, N. Peter Wiklund, Fredrik Wiklund, Henrik Grönberg

Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

Background: Nitric-oxide synthase (NOS)-polymorphisms influence the cellular amount of NO, and are associated with disease-risk in many disorders. We investigated 145 SNP-polymorphisms and a (CCTTT)_n-microsatellite in the NOS2-gene in 3161 prostate-cancer patients and 2149 controls from a Swedish population-based GWAS-study.

Aim: To analyze possible associations between NOS2-polymorphisms, prostate cancer, and prostate cancer pathogenesis.

Methods: Two groups were analyzed, those with advanced tumours (Gleason_≥6), and those with tumours of mixed Gleason-statuses. Affymetrix 5.0-chip (SNP-polymorphisms), DNA Fragment-analysis and Sequencing ((CCTTT)_n-microsatellite) were used for genotyping. Genotypes were combined with information on tumour stage, Gleason, PSA, metastases and cancer-specific death, using clinical follow-up.

Results: We divided the (CCTTT)_n-alleles into short (S, $n \leq 10$), intermediate (M, $n = 11-12$) and long (L, $n \geq 13$). Patients homozygous for longer repeats (LL) had decreased risk of highly aggressive (Gleason ≥ 7 ; PSA > 20 ; T3+) tumours (OR:0.40; CI:0.14-1.08; $p=0.071$), but, once ill they showed a threefold increased risk of dying in prostate cancer (HR:3.31; CI:0.85-12.85; $p=0.084$), compared to SS-homozygotes. The SNP-alleles that co-varied with the (CCTTT)_L-allele also had lower risk of aggressive tumours, as well as, once ill, a 2-4 times higher risk of dying ($p=0.009$). Also the proportion of patients with lymph node metastases increased with length of the (CCTTT)_n-alleles of the patients (SS < SM < SL < MM < ML < LL)(trend analysis; $p=0.033$).

Conclusions: Nitric oxide can induce proliferation as well as apoptosis depending on cellular context. Our results suggest that NOS2 polymorphisms may influence the risk of aggressive prostate cancer and that these polymorphisms could have an impact on disease pathogenesis, possibly by affecting intracellular nitric oxide levels.

<http://dx.doi.org/10.1016/j.redox.2015.09.029>