Preface: First International Conference on Nitrosylation in Oncology and Immunology; Speakers’ Presentations

The First International Conference on Nitrosylation in Oncology and Immunology was held February 2–3, 2012, in Dijon, France. This conference was organized as a result of recent interest in and various publications about the important role of protein nitrosylation in the regulation of cell function and cancer. S-nitrosylation has been implicated in numerous physiological processes such as synaptic transmission, vasodilation, angiogenesis, and inflammation. It participates in cellular trafficking and cell signaling, which affect cell motility, proliferation, differentiation, and death. The consequences of deficient or excessive nitrosylation have been implicated in numerous diseases and particularly in carcinogenesis, tumor progression, and tumor metastasis. S-nitrosylation is one of the main activities of nitric oxide (NO) and depends on both NO synthases and denitrosylases.

This conference lasted 2 days and covered a wide spectrum of topics relevant to the main goals of the conference. There were 6 sessions, each of which consisted of more than one presentation. The first session, “New NO Donors and S-Nitrosylation,” covered 2 subjects. 1) Dr. K. Kashfi (University of New York) presented “Nitric oxide–releasing hybrid drugs target cellular processes through S-nitrosylation.” He and his colleagues examined several hybrids that release NO (NO–nonsteroidal anti-inflammatory drugs, NONO–nonsteroidal anti-inflammatory drugs, and JSK ) and demonstrated that several of these inhibited nuclear factor (NF)-κB activity and activated caspase 3, leading to cell death by apoptosis. Both NF-κB and β-catenin were S-nitrosylated, and rats treated with NO hybrids showed several responses correlating with their chemopreventive activities. 2) Dr. L. Keefer (National Cancer Institute, Frederick, Maryland) presented “Thiol modification by pharmacologically active agents of the diazeniumdiolate class.” He described several newly synthesized drug candidates of the diazeniumdilates (NONOate) family, which mediate several types of thiol modification. The NO generated as a byproduct can undergo oxidation, leading to S-nitrosylation and S-glutathionylation.

Session 2 dealt with “S-Nitrosylation, Molecular Targets, Structural and Functional Effects,” and 4 speakers presented their findings. 1) Dr. H. Ischiropoulos (University of Pennsylvania, Philadelphia) presented “Structural and functional diversity of protein S-nitrosylation.” He used mass spectrometry–based approaches that revealed 787 unique S-nitrocysteine sites mapped on 468 proteins from different organs in wild mice. More than 50% of the S-nitrocysteine residues were dependent on NO derived from endothelial NO synthase (eNOS). His findings also showed that S-nitrosylation was involved in the regulation of liver mitochondrial β-oxidation of fatty acids and is a potential mediator of non-alcoholic fatty liver, a common human disorder. 2) Dr. M. Benhar (Israel Institute of Technology, Haifa, Israel) presented “Proteomic strategies for identifying S-nitrosylated targets of thioredoxin.” His findings deal with the mechanisms of denitrosylation of protein cysteine residues by specific cysteine denitrosylases, namely, S-nitrosoglutathione (GSNO) reductases and thioredoxin. He presented novel approaches to identify S-nitrosylated targets of thioredoxin.
that are involved in a wide range of cellular functions such as cytoskeletal organization, cellular metabolism, signal transduction, and redox homeostasis. 3) Dr. B. Mutus (University of Windsor, Windsor, Canada) presented “Does S-nitrosylation of neutral sphingomyelinase control plasma membrane cholesterol in cancer cells?” He presented findings on endothelial cells under endoplasmic reticulum stress led to the inactivation of neutral shingomyelinases, which resulted in the elevation of cholesterol levels and attenuation of NO production. These findings suggested the role of cholesterol levels, obesity, and hypertension in both the incidence and progression of cancer. 4) Dr. Y. Chen (Institute of Chemistry, Academia Sinica, Taiwan) presented “Decoding the personalized tissue S-nitrosoproteome in human colorectal cancer.” He discussed an integrated quantitative proteomic approach combining an S-alkylating biotin-switch method and label-free approaches for site-specific identification and quantitation of the S-nitrosoproteome. Higher S-nitrosylated levels were found in tumor tissues compared with the adjacent normal tissues in colorectal cancer.

The third session of the conference covered "S-Nitrosylation, Epithelial to Mesenchymal Transition, and Tumor Cell Death.” Dr. Leon-Bolotte presented “GTN sensitises tumor cells to apoptosis: implication of S-nitrosylation,” and discussed NO-mimetic nitroglycerin glyceryl trinitrate–induced apoptotic activities in cancer cells through the S-nitrosylation of cysteine residues 199 and 304 in the cytoplasmic domain of the Fas receptor, resulting in its recruitment into lipid rafts and tumor cell sensitization to Fas ligand apoptosis. Dr. Rojanasakul presented “S-nitrosylation of caveolin-1 regulates lung carcinoma cell anoikis.” Anoikis (detachment-induced apoptosis) is a mechanism of the inhibition of tumor cell metastasis, although the mechanism is unknown. He demonstrated that NO impairs the anoikis function of cells via a mechanism that is dependent on S-nitrosylation of caveolin-1. Resistance to anoikis is mediated by downregulation of caveolin-1 during cell detachment. NO inhibited this downregulation by interfering with caveolin-1 ubiquitination through S-nitrosylation. Bonavida presented “Inhibition of epithelial to mesenchymal transition (EMT) in cancer by NO: pivotal roles of nitrosylation of NF-κB, YY1, and Snail.” The findings demonstrated the S-nitrosylation of proteins that regulate the EMT. The EMT phenotype can be induced through dysregulation of the NF-κB/YY1/Snail/RKIP circuit. The activation of NF-κB and its targets, the metastasis-inducer Snail, and YY1 result in the inhibition of the metastasis suppressor RKIP, and all of these lead to EMT. Treatment of EMT+ cancer cells with NO resulted in the inhibition of EMT via S-nitrosylation of NF-κB (P50, 65), Snail, and YY1 and the induction of RKIP. The findings suggested the therapeutic potential of NO donors in the inhibition of EMT and metastasis.

Session 4, “S-Nitrosylation, Mutagenesis, Carcinogenesis, Tumor Promotion, and Tumor Growth,” had 4 speakers. Dr. L. Liu (University of California, San Francisco) presented “S-nitrosylation from GSNOR deficiency promotes mutagenesis and hepatocarcinogenesis,” discussing the level and activity of GSNO reductase (GSNOR), which is critical for protein S-nitrosylation, and showed a significant decrease of GSNOR in 50% of patients with hepatocellular carcinoma. GSNOR-deficient mice are susceptible to both carcinogen-induced and spontaneous hepatocellular carcinoma. Dr. D. Wink (National Cancer Institute, Bethesda, Maryland) presented “The role of discrete levels of NO, nitrosation, and iNOS in tumor promotion in cancer.” He discussed the role of inflammation-generated reactive chemical species that induced conditions of oxidative nitrosative stress in relation to cancer. Inflammatory protein-inducing NO synthase has been shown to be a strong predictor of poor outcome in endoplasmic reticulum+ patients. Numerous
genes have been associated with basal-like breast cancer and were shown to be elevated when inducible NO synthase (iNOS) was high. Dr. J. P. Gratton (Université de Montreal, Montreal, Canada) presented “eNOS and nitric oxide signalling at endothelial cell junctions: implications in tumor angiogenesis.” He showed that the release of NO from endothelial cells was essential for the proangiogenic and permeability effects of vascular endothelial growth factor. To investigate the mechanism of an NO-induced increase in endothelial permeability, Dr. Gratton investigated the direct effect of NO adherens junction complexes that are responsible for interendothelial cell contacts, showing that NO covalently modifies β-catenin through cysteine S-nitrosylation, which results in the disassembly of the adherens junction complexes and increased endothelial permeability. Dr. H. Monteiro (Universidade Federal de São Paulo, São Paulo, Brazil) presented “A role for nitric oxide and for inducible nitric oxide synthase in tumor biology.” He discussed the use of human primary colon cancer cell lines SW480 and the metastatic SW620 derived from the same patient metastatic site. SW620 expresses high levels of iNOS, and knockdown of iNOS results in decreased levels of proteins associated with apoptosis, cell proliferation, and drug resistance.

Session 5, “S-Nitrosylation and Tumor Risk, Prevention, and Therapy,” included 2 presentations. Dr. B. Gaston (University of Virginia, Charlottesville) presented “S-nitrosylation and lung cancer risk.” He described data related to the role of N-glutathione reductase as a Ras denitrosylase in human lung cancer. Wild-type Ras is S-nitrosylated and activated by nitrosative stress and is denitrosylated by GSNOR. In human lung cancer, GSNOR activity and expression are decreased. Dr. C. Rao (University of Oklahoma) “Targeting iNOS for Cancer Prevention and Treatment: Where we stand?” He examined the role of NO in stimulating cyclooxygenase-2 activity and tumor invasiveness. He examined iNOS inhibitors (PBIT and NILT) in rats with invasive colonic adenocarcinoma, showing increased efficacy of the combination of iNOS inhibitors and celecoxib (a cyclooxygenase-2 inhibitor) in the inhibition of invasiveness.

Session 6, “S-Nitrosylation in Immune Response,” included a presentation by Dr. H. Marshall (Duke University, Durham, North Carolina) titled “Regulation of the immune response by S-nitrosylation of NF-κB.” Using a mouse model of acute lung injury, Dr. Marshall presented data showing that the NF-κB pathway regulates many genes in the immune response. Both subunits of the NF-κB heterodimer are targeted by S-nitrosoylation and regulate several immune response gene products. Both subunits of the NF-κB heterodimer are targeted by S-nitrosylation and regulate several immune response gene products. Dr. A. Martinez-Ruiz (Instituto de Investigación Sanitaria Princesa, Madrid, Spain) presented “Thiol redox proteomics and S-nitrosylation in the immune synapse.” He discussed data on the role of S-nitrosylation in the decrease of macrophage activity as well as the role of S-nitrosylation of N-Ras on T cells.

These presentations (several of which are published in this volume) clearly highlighted the current status of both the function and activity of S-nitrosylation in the regulation of cancer. The findings provided new insights and directions for future studies of the role of nitrosylation of proteins in various physiological processes and diseases including cancer. It is hoped that future studies will generate agents that can selectively interfere with specific nitrosylated proteins as targets for intervention.

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