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.

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

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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.

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

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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.

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Young Investigation Session Selected Oral Communications

Nitric Oxide And Hypoxia Response In Pluripotent Stem Cells

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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