Protein kinase G type-I phosphorylates c-Src at serine-17 and promotes cell survival, proliferation and attachment in human mesothelioma and non-small cell lung cancer cells

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From 5th International Conference on cGMP: Generators, Effectors and Therapeutic Implications Halle, Germany. 24-26 June 2011

Background
Previously, we demonstrated that protein kinase G type-1α (PKG-Iα) plays an important role in promoting cell survival in neural cells (N1E-115 neuroblastoma and NG108-15 neuroblastoma-glioma hybrid cells) and significantly contributes to the serine-155 phosphorylation of BAD, an apoptosis-regulating protein [1]. We also found that PKG-Iα promotes cell survival and proliferation in mouse OP9 bone marrow stromal cells [2] and human ovarian cancer cells [3] (determined by using both pharmacological inhibitors (ODQ, DT-2 and DT-3) and gene knockdown (siRNA) to reduce PKG-Iα/β activity). In the case of ovarian cancer cells [3] (determined by using both pharmacological inhibitors (ODQ, DT-2 and DT-3) and gene knockdown (siRNA) to reduce PKG-Iα/β activity), the pro-growth and pro-survival effects involved a novel interaction between PKG-Iα and the oncogenic protein c-Src [3]. For example, intracellular activation of PKG-Iα, assessed by VASP serine-239 phosphorylation, was dependent on c-Src-catalyzed tyrosine phosphorylation of PKG-Iα (i.e. VASP phosphorylation was blocked by Src inhibitors, SKI-1 or SU6656) and the intracellular activation of c-Src was (somehow) dependent on the kinase activity and expression levels of PKG-Iα (i.e. c-Src activation was decreased by DT-2 or siRNA-induced knockdown of PKG-Iα).

Hypothesis, research plan and methods
We hypothesize that c-Src activation in cancer cells involves the PKG-Iα-catalyzed phosphorylation of a serine or threonine residue of c-Src, likely serine-17 (because amino acid residues around the serine-17 site of c-Src provide a good consensus sequence for PKG-Iα/β).

Results
In in vitro incubations using recombinant human c-Src and either PKG-Iα or PKG-Iβ, both recombinant PKG-Iα and PKG-Iβ were able to directly catalyze the phosphorylation of serine-17 in c-Src, resulting in increased autophosphorylation of c-Src at tyrosine-419 (equivalent to tyrosine-416 in mouse c-Src). The NSCLC cells (NCI-H23...
lung cancer cells) were found to express only the PKG-Iα isoform, whereas both of the mesothelioma cell lines (MSTO-211H and NCI-H2052) express both PKG-Iα and PKG-Iβ. In all cell lines, inhibition of PKG-Iα/β kinase activity using DT-2 or silencing of PKG-Iα/β expression using shRNA dramatically reduced the intracellular phosphorylation of c-Src at serine-17, whereas addition of the PKG activator 8-bromo-cGMP increased c-Src phosphorylation at serine-17. Both the kinase-activity inhibition and knockdown of PKG-Iα/β caused significant increases in apoptosis (i.e. decreases in cell survival) and dramatically decreased the cell proliferation, colony formation and cell attachment in both the lung cancer and mesothelioma cells.

Conclusion
The mesothelioma cells in this study express both PKG-Iα and PKG-Iβ, and the inhibition of their expression or kinase activity results in dramatic suppression of the phosphorylation of c-Src at serine-17 and the endogenous activation of c-Src as well as the cell survival, proliferation and attachment of these cells. Because the kinase inhibitors and shRNA constructs are (at present) not able to discriminate between the two PKG-I isoforms, it is not yet possible to identify which PKG-I isoform is mediating the c-Src phosphorylation and the pro-survival, pro-growth and pro-attachment effects of PKG-I in mesothelioma cells. In the NCI-H23 lung cancer cells, which express only the PKG-Iα isoform, the PKG-Iα was identified as a key protein kinase mediating the enhanced phosphorylation and activation of c-Src and the downstream enhancement of cell survival (contributing to chemoresistance), proliferation and attachment of these lung cancer cells. This builds upon our previous observation that c-Src kinase activity promotes tyrosine-phosphorylation and activation of PKG-Iα in ovarian cancer cells. Thus, it appears that the interactions between c-Src and PKG-I in cancer cells may represent a novel “oncogenic re-enforcement”, with each protein kinase phosphorylating and enhancing the kinase activity of the other, ultimately contributing to chemoresistance and tumor growth. This interaction between c-Src and PKG-I may provide a novel target for the development of new anticancer therapeutic agents.

References
1. Johlfs MG, Fiscus RR: Protein kinase G type-Iα phosphorylates the apoptosis-regulating protein Bad at serine 155 and protects against apoptosis in N1E-115 cells. Neurochem Int 2010, 56:546-553.
2. Wong JC, Fiscus RR: Essential roles of the nitric oxide (NO)/cGMP/protein kinase G type-Iα (PKG-Iα) signaling pathway and the atrial natriuretic peptide (ANP)/cGMP/PKG-Iα autocrine loop in promoting proliferation and cell survival of OP9 bone marrow stromal cells. J Cell Biochem 2011, 112:829-839.
3. Leung EL, Wong JC, Johlfs MG, Tsang BK, Fiscus RR: Protein kinase G type-Iα activity in human ovarian cancer cells significantly contributes to enhanced Src activation and DNA synthesis/cell proliferation. Mol Cancer Res 2010, 8:578-591.

Published: 1 August 2011

Cite this article as: Fiscus and Johlfs: Protein kinase G type-Iα phosphorylates c-Src at serine-17 and promotes cell survival, proliferation and attachment in human mesothelioma and non-small cell lung cancer cells. BMC Pharmacology 2011 11(Suppl 1):O31.

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