Memo: Is Peroxynitrite a Mediator of Survival and Aggressive Growth in Pancreatic Adenocarcinoma?

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There are a number of reports that NADPH oxidase activity is elevated in human pancreatic ductal adenocarcinomas (PDAs), or cell lines derived therefrom, and that the resultant production of superoxide protects the cancer from apoptosis.[1-6] Recently, Du and colleagues have reported that oncogenic mutants of K-Ras – expressed in over 90% of such cancers – promote NADPH oxidase activity via induction of Nox2.[4;6] In other PDA cell lines, Nox2 has not been detected, but other isoforms of NADPH oxidase, such as Nox4, are expressed.[3;7] Growth factors for PDAs have been shown to boost their NADPH oxidase activity.

It would be logical to suspect that the hydrogen peroxide downstream from NADPH oxidase-generated superoxide would boost growth factor activity by inhibiting certain tyrosine phosphatases that oppose the activation of growth factor receptors; indeed, there is some evidence that this phenomenon may be operative in PDAs.[2] However, Du's work indicates that superoxide, rather than hydrogen peroxide, may be the chief mediator of NADPH oxidase's impact on PDA survival.[6] Thus, transfection with the cytoplasmic form of superoxide dismutase, or treatment with the SOD mimetic drug tempol, was shown to inhibit survival of a tumorigenic pancreatic ductal cell line expressing K-Ras. This effect would not be expected if hydrogen peroxide were the key mediator of NADPH oxidase's favorable impact on PDA survival.

How then could superoxide promote survival in PDAs? Hydroxyl radical generated via the Haber-Weiss reaction might conceivably mediate the anti-apoptotic impact of superoxide in PDA, but this is counterintuitive; hydroxyl radical, with its non-specific destructive effects, would seem more likely to kill PDA cells than rescue them. A more reasonable prospect is that peroxynitrite might mediate superoxide's effect in this regard. Indeed, a very marked increase in tyrosine nitration has been observed in human PDAs, as compared to normal pancreatic ductal tissue; activation of c-Src via tyrosine nitration has been suggested to play a role in PDA growth.[8] Moreover, eNOS has been observed in PDAs, and this eNOS is activated via K-Ras/PI3K/Akt signaling.[9;10] Knockdown of eNOS expression in PDA cell lines markedly decreases their growth potential.[9] Similarly, administration of the NOS inhibitors L-NAME or aminoguanidine has been shown to slow the growth and spread of human PDAs implanted in nude mice.[10;11] Lampson and colleagues have recently suggested that pharmacological inhibition of eNOS may be a viable strategy for control of PDA.[10] There is evidence that S-nitrosylation of NRas and HRas promotes the growth of PDAs by stimulating dissociation of GDP from these G-proteins, enhancing their activation.[9;12] However, this does not rule out the possibility that NO might also promote PDA survival via peroxynitrite.

The possibility that activation of c-Src via tyrosine nitrosylation might be a mediator of PDA's survival and spread is consistent with evidence that this protein is overexpressed in most PDAs, and that inhibition of the expression or activity of c-Src suppresses the growth, metastatic spread, and chemoresistance of PDAs.[13-17] It would be interesting to know whether suppression of c-Src activity decreases the modulatory impact of superoxide and NO on PDA survival.

Other mechanisms whereby peroxynitrite-mediated tyrosine nitration might contribute to cancer survival and aggressiveness include inactivation of IkappaBα, suppression of the DNA binding activity of p53, and inhibition of tissue inhibitor of metalloproteinase-4.[18-20] Peroxynitrite also promotes activation of matrix metalloproteinases, aiding tissue invasion.[21] And peroxynitrite could aid evolution of chemoresistance by increasing the genetic lability of cancer.

If peroxynitrite, stemming from joint activation of NADPH oxidase and eNOS, is indeed a key mediator of PDA survival and spread, practical strategies for controlling PDA may be at hand. Phycocyanobilin (PhyCB), richly supplied by cyanobacteria such as spirulina, has recently been shown to mimic bilirubin's capacity to inhibit certain isoforms of NADPH oxidase; in rodents, this effect can be achieved via oral administration of whole spirulina or of phycocyanin, the spirulina protein which contains PhyCB as a covalently-bound chromophore.[22;23]

Whereas it might be clinically feasible to inhibit eNOS with L-NAME, as suggested by Lampson and colleagues, this would doubtless entail increased cardiovascular risk (albeit this would presumably be more acceptable in patients with PDA than it would be in healthy subjects). An alternative approach would be to administer agents which scavenge peroxynitrite. Certain drugs with this capacity have been studied in rodent models, but are not clinically available. Most intriguingly, reduced metabolites of folic acid have been found to be effective scavengers for peroxynitrite, and high concentrations of these metabolites can be achieved within cells when high doses of folic acid are administered.[24-26] Indeed, this phenomenon may explain the ability of high-dose folate to prevent the peroxynitrite-mediated uncoupling of eNOS in oxidatively stressed vascular endothelium.[25;27] High-dose folate is also notably protective in a rat model of cardiac ischemia-reperfusion damage, likely reflecting its potent antioxidant activity.[28] Dr. Kurt Oster long treated angina patients with high-dose folate (40-80 mg daily), without noting any adverse effects, and recent clinical studies suggest that this practice may indeed be beneficial in angina.[29-31] Hence, it would be intriguing to determine whether supraphysiological concentrations of folic acid might inhibit PDA survival by opposing the effects of peroxynitrite.

If these speculations prove valid, joint administration of ample doses of spirulina and folic acid might have clinical potential as a safe nutraceutical regimen for slowing growth and spread of PDA. Such a combination might be particularly appropriate since safe and feasible intakes of PhyCB will produce at best only a partial inhibition of NADPH oxidase activity.

Finally, it may be noted that avid superoxide production by PDAs may render them sensitive to ascorbate-mediated killing. High-dose parenteral sodium ascorbate retards the growth of certain human PDAs implanted in nude mice, and complements the efficacy of gemcitabine in this regard.[32-35] This strategy promotes the production of hydrogen peroxide in the tumor extracellular matrix, and this hydrogen peroxide mediates the lethal impact of ascorbate infusion.[36;37] The selective susceptibility of certain cancers, including PDAs, to killing by ascorbate-generated hydrogen peroxide has not been clearly explained. However, Ranzato and colleagues have recently shown that the susceptibility of mesothelioma to killing by ascorbate reflects its NOX4-mediated production of superoxide; apocynin and NOX4 small interfering RNA alleviated the toxic impact of ascorbate on this cancer.[38] The authors credibly suggest that a Haber-Weiss reaction, in which extracellularly-generated hydrogen peroxide reacts with endogenously produced superoxide via metal catalysis to engender highly toxic hydroxyl radicals, may mediate the lethal impact of ascorbate in this system; they cite a previous study in which pre-treatment

with a cell-permeant metal chelator was found to protect cancer cells from ascorbate toxicity.[39] A similar phenomenon might explain the high susceptibility of many pancreatic cancer lines to ascorbate-mediated killing. Hence, the high NADPH oxidase activity characteristic of PDAs may render them selectively susceptible both to killing by intravenous sodium ascorbate, and to growth retardation or chemosensitization induced by the feeding of spirulina – though these measures evidently should not be used simultaneously.[40]

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