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Research Paper

Intracellular redox status controls membrane localization of pro- and anti-migratory signaling molecules ☆

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ABSTRACT

Shifts in intracellular Reactive Oxygen Species (ROS) have been shown to contribute to carcinogenesis and to tumor progression. In addition to DNA and cell damage by surges in ROS, sub-lethal increases in ROS are implicated in regulating cellular signaling that enhances pro-metastatic behavior. We previously showed that subtle increases in endogenous H_2O_2 regulate migratory and invasive behavior of metastatic bladder cancer cells through phosphatase inhibition and consequential phosphorylation of p130cas, an adapter of the FAK signaling pathway. We further showed that enhanced redox status contributed to enhanced localization of p130cas to the membrane of metastatic cells. Here we show that this signaling complex can similarly be induced in a redox-engineered cell culture model that enables regulation of intracellular steady state H_2O_2 level by enforced expression of superoxide dismutase 2 (Sod2) and catalase. Expression of Sod2 leads to enhanced p130cas phosphorylation in HT-1080 fibrosarcoma and UM-UC-6 bladder cancer cells. These changes are mediated by H_2O_2, as co-expression of Catalase abrogates p130cas phosphorylation and its interaction with the adapter protein Crk. Importantly, we establish that the redox environment influence the localization of the tumor suppressor and phosphatase PTEN, in both redox-engineered and metastatic bladder cancer cells that display endogenous increases in H_2O_2. Importantly, PTEN oxidation leads to its dissociation from the plasma membrane. This indicates that oxidation of PTEN not only influences its activity, but also regulates its cellular localization, effectively removing it from its primary site of lipid phosphatase activity. These data introduce *hitherto* unappreciated paradigms whereby ROS can reciprocally regulate the cellular localization of pro- and anti-migratory signaling molecules, p130cas and PTEN, respectively. These data further confirm that altering antioxidant status and the intracellular ROS environment can have profound effects on pro-metastatic signaling pathways.

Introduction

Redox signaling is implicated in regulating a diverse range of cellular functions. In diseases, such as cancer, sub-lethal increases in intracellular ROS have been associated with aberrant signaling that exasperates pathophysiological phenotypes. It has been shown that metastatic tumor cells display elevated in intracellular ROS and alterations in their antioxidant status [1–3]. For example, many aggressive cancers display increased mitochondrial manganese superoxide dismutase (Sod2) levels and loss of catalase (CAT) expression that are associated with stage and grade of disease progression and poor patient outcome. This shift in antioxidant enzyme levels may be an intrinsic adaptation of metastatic cancer cells to cope with changes in intracellular ROS [3]. A number of studies indicate that metastatic tumor cells have the capability to utilize these increases in ROS to regulate transcription and cellular signaling that contribute to metastatic disease progression [4–7].

We have developed number of cell culture models, which either display endogenous increases in intracellular ROS as a result of acquisition of the metastatic phenotype or through manipulation of antioxidant enzyme expression. We have observed that increased intracellular ROS levels can drive migration, invasion and metastasis *in vitro* and *in vivo*, and have begun to elucidate the redox-sensitive molecular triggers that accompany the metastatic phenotype [7–9]. Using a bladder cancer cell culture model of metastatic disease progression we have shown that a highly metastatic cell line derivative (253J-BV) relies on redox-mediated signaling to drive its migratory and invasive phenotype compared to a related non-metastatic parental cell line [7,10]. Subtle increases in the ROS milieu have been shown to have profound effects on pro-metastatic signaling of the Focal Adhesion Kinase (FAK) pathway by

Abbreviations: CAT, catalase; FAK, focal adhesion kinase; H_2O_2, hydrogen peroxide; MMP, matrix metalloproteinase; Nox, NADPH oxidase; p130cas, Crk-associated substrate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homolog; PTP, protein tyrosine phosphatase; ROS, reactive oxygen species; Sod2, manganese superoxide dismutase

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enhancing signaling through the adapter protein p130cas (Crk-associated substrate) which links FAK to Rac-1 activation and cytoskeletal rearrangement during the process of migration and focal adhesion formation [7]. Redox regulation of signaling occurs primarily through reversible oxidation of proteins, which is particularly prevalent in the case of phosphatases, where active site cysteine residues are susceptible to oxidation due to their relatively low pKa [11]. Increases in endogenous steady state H_2O_2 enhance oxidation of the phosphatase pool, p130cas function and migratory activity of the metastatic 253J-BV bladder cancer cells through the oxidative inhibition the p130cas inhibitory phosphatase, PTP-N12 [7]. In addition, ROS play a pivotal role in oxidative inactivation of the dual protein and lipid phosphatase PTEN (phosphatase and tensin homolog), leading to redistribution of the phosphatidylinositol (3,4,5)-trisphosphate (PIP3) pools at the plasmalamellar membrane, enhancing Akt signaling and tumor cell migration [12–14].

In the present study we show that redox-regulation of p130cas signaling is not limited to metastatic bladder cancer cells, but can similarly be induced by altering steady state H_2O_2 levels following manipulation of Sod2 and CAT expression. We previously used this model to show that Sod2-mediated changes in steady state H_2O_2 lead to an enhanced metastatic and angiogenic phenotype through PTEN oxidation and increased VEGF expression. We have also established that the Sod2-dependent increases in H_2O_2 drive high level expression of the matrix metalloproteinase MMP-1 that often accompanies metastatic disease progression [8,9,13,15]. By modulating steady state H_2O_2 levels using well characterized redox engineered fibrosarcoma cell lines [16], we observed similar changes in redox dependent p130cas phosphorylation and its membrane recruitment. Further, using this and a bladder cancer progression model, we demonstrate that intracellular redox status influences both PTEN oxidation and its cellular distribution. Oxidation moved PTEN away from its primary site of action at the cell membrane to the cytosol. These data show that increases in steady state H_2O_2 levels can have essentially opposing actions on membrane recruitment of pro- and anti-migratory signaling players, ultimately supporting a redox-dependent pro-metastatic signaling complex at the leading edge of metastatic tumor cells.

Results

Sod2 expression leads to H_2O_2-mediated p130cas phosphorylation

We previously showed that increases in both endogenous and exogenous-applied H_2O_2 can stimulate p130cas phosphorylation in 253 bladder cancer cells [7]. Analysis of redox engineered HT-1080 cells [16] revealed that Sod2 overexpression similarly enhanced p130cas phosphorylation, which was reversed by CAT co-expression (Fig. 1A). To confirm this finding, stable expression of Sod2 in another bladder cancer cell line UM-UC-6, enhanced phosphorylation of p130cas and FAK (Fig. 1B). Treatment of UM-UC-6 cells with exogenous CAT was also able to reverse these effects, suggesting that increases in H_2O_2 in response to Sod2 expression play a significant role in regulating phosphorylation of p130cas. In addition, Sod2 expression markedly enhanced Crk association with p130cas, which is dependent on p130cas phosphorylation and necessary for p130cas down-stream signaling. This association was again abrogated by CAT co-expression (Fig. 1C). These data show that modulating antioxidant enzyme levels similar to that observed in metastatic cells, can activate redox-dependent signaling networks that engage migratory activity.

Loss of p130cas prevents cell migration

We previously showed that Sod2 expression enhances migration and invasion of HT-1080 tumor cells [9]. To assess if p130cas is a

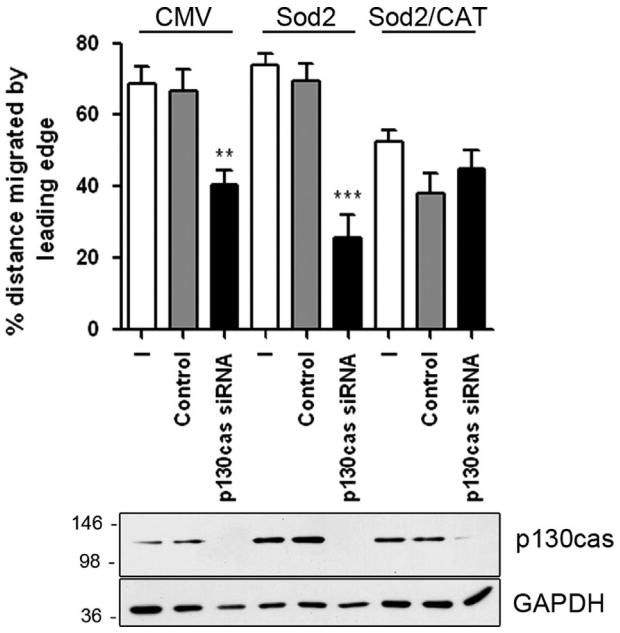

Fig. 2. p130cas knock-down significantly abrogates migration of HT-1080 redox engineered cells (stable-transfected with empty vector CMV, Sod2 or Sod2/CAT) in a wound healing assay. Cells were mock transfected (-) or with siRNA construct against p130cas or scramble control and allowed to reach a confluent monolayer. Migration of cells in serum-free media into the scratch wound was monitored for 23 h. Percentage of the distance migrated by the leading edge was quantified ($n=7$; mean ± SEM, t-test, $^{**}p<0.01$, $^{***}p<0.001$, compared to scramble control).

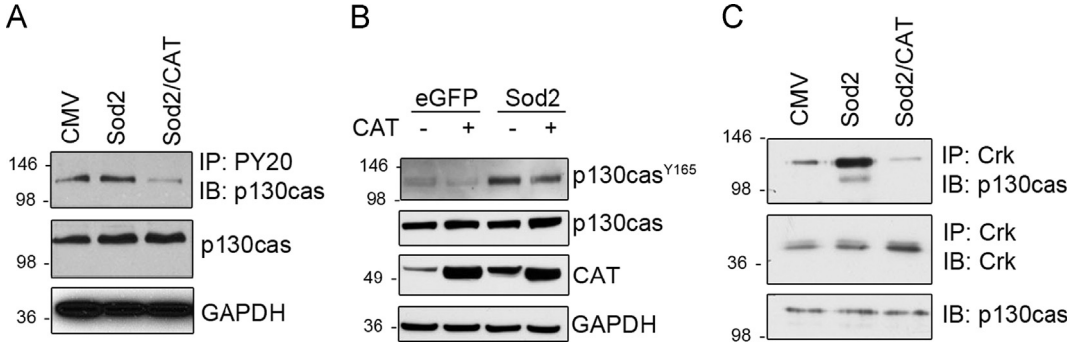

Fig. 1. Sod2-mediated shifts in redox status regulate p130cas phosphorylation. (A) p130cas phosphorylation profile of redox engineered HT-1080 cells. Cells were stably transfected with vector (CMV), Sod2 or Sod2 and CAT (Sod2/CAT) and cell lysates immunoprecipitated (IP) for phospho-tyrosine (PY-20), followed by immunoblotting (IB) for p130cas. (B) Increased Sod2 expression enhances p30cas phosphorylation in UM-UC-6 bladder cancer cells, which is reversed by CAT treatment. UM-UC-6 cells stably transfected with vector only (eGFP) or Sod2-eGFP. Cells were treated with recombinant CAT (500 U/ml) to assess H_2O_2 dependence of p130cas phosphorylation. (C) Sod2-expressing redox-engineered cells display enhanced Crk-p130cas interaction. Cells were lysed using RIPA buffer, followed by IP with antibody against Crk and immunoblotting with indicated antibodies. CAT co-expression abrogates association of Crk with p130cas (Sod2/CAT).