FORUM REVIEW ARTICLE

Renal Carcinogenesis, Tumor Heterogeneity, and Reactive Oxygen Species: Tactics Evolved

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Abstract

Significance: The number of kidney cancers is growing 3–5% each year due to unknown etiologies. Intra- and inter-tumor mediators increase oxidative stress and drive tumor heterogeneity. **Recent Advances:** Technology advancement in state-of-the-art instrumentation and methodologies allows researchers to detect and characterize global landscaping modifications in genes, proteins, and pathophysiology patterns at the single-cell level. **Critical Issues:** We postulate that the sources of reactive oxygen species (ROS) and their activation within subcellular compartments will change over a timeline of tumor evolution and contribute to tumor heterogeneity. Therefore, the complexity of intracellular changes within a tumor and ROS-induced tumor heterogeneity coupled to the advancement of detecting these events globally are limited at the level of data collection, organization, and interpretation using software algorithms and bioinformatics. **Future Directions:** Integrative and collaborative research, combining the power of numbers with careful experimental design, protocol development, and data interpretation, will translate cancer biology and therapeutics to a heightened level or leave the abundant raw data as stagnant and underutilized. *Antioxid. Redox Signal.* 25, 685–701.

Keywords: tumor heterogeneity, oxidative stress, NOX oxidases, mitochondrial electron transfer chain, renal cancer, high-throughput analysis

Introduction

EPIDEMIOLOGICAL EVIDENCE SUGGESTS that renal cancer is on the rise whereas the death rate has not declined. According to the American Cancer Society, ~64,000 new cases of kidney cancer are diagnosed in the United States alone, a number that has been increasing by 3% per year for the past 5 years. One-third of those affected will die. Although there is a predisposition of men over women to be diagnosed with renal cancer, molecular insights have not detected major differences in the preponderance of a specific histological subtype or the underlying biology of the tumor. However, our ability to target renal cancer with drug therapy remains scarce, suggesting a strong role for environmental or other risk factors. Technology to detect and elucidate molecular changes in kidney cancer at the single-cell level has improved over the past decade. In part, the ability to isolate single cells, perform high-throughput biology analysis (gene expression, methylation profiling, microRNA [miRNA] expression, me-

tabolomics etc...), and interpret the data *via* algorithms and bioinformatics is giving a new broad overview of changes in pathway landscaping at the single-cell level. However, caution of data interpretation must heed to the affects of tumor heterogeneity, including genetic variation and the effects of tumor environmental cues such as nutrient and oxygen variability, not to mention metabolic syndrome-produced effects of oxidative stress, growth factors, insulin, glucose, hormones, and inflammatory cytokines. This article will examine current molecular and biochemical understandings of renal cell carcinoma (RCC) and the role of metabolic syndrome and tumor environment in tumor heterogeneity, metastasis, and drug resistance.

Histological Classification and Renal Cancer

Introduction

In large part, the function of the kidney is to filter and remove water-soluble waste from the blood in the form of

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urine and to reabsorb water, glucose, and electrolytes. The kidney is a complex organ comprising an outer renal cortex and an inner renal medulla. Nephrons, the functional structures of the kidney, span the cortex and medulla. One nephron anatomically comprises the glomerulus, proximal tubule, loop of Henle, distal convoluted tubule, and collecting duct. Renal cancer arises from different parts of the nephron and is classified histologically using the World Health Organization classification of genitourinary tumor subtypes. There are four major histological subtypes of cortical RCC: (i) clear cell carcinoma, also known as conventional; (ii) papillary (derived from proximal tubules); (iii) chromophobe; and (iv) collecting duct (derived from the collecting duct). Histological grading using the Fuhrman system assesses microscopic morphology and hematoxylin and eosin staining, where Grade I (early-stage tumors) nuclei are round, uniform, and $\sim 10\ \mu\text{m}$ and Grade IV (advanced tumors) nuclei are bizarre, multilobated, and $20\ \mu\text{m}$ or larger with nucleoli present. Staging renal cancer is important for determining prognosis and is based on the TNM (T=tumor, N=lymph nodes, M=metastasis) staging or the American Joint Committee on Cancer (AJCC) (Stage I–IV) system, where Stage I is less than 7 cm and is limited to the kidney and Stage IV includes large spreads, involvement of lymph nodes, and/or has distant metastasis. RCC can be divided into adenomas and carcinomas, where Bells' rule states the following: ($<3\text{ cm}$: adenoma; $>3\text{ cm}$: carcinoma). Figure 1 overlays the RCC histological subtypes derived from the anatomical cell type of the kidney.

Conventional Clear Cell Carcinoma (70–80% of all RCC)

Clear cell renal carcinoma, also known as conventional carcinoma, is histologically the most common form of renal cancer and is likely derived from the proximal renal tubular epithelium. Conventional renal cancer is histologically classified as clear cells due to rich lipid and glycogen buildup in the cytoplasm (characteristic clear cytoplasm). Inherited or

sporadic mutations of the von Hippel–Lindau tumor suppressor gene (*VHL*) give rise to renal clear cell carcinoma (51, 75, 118). The *VHL* gene encodes the pVHL protein, an E3 ubiquitin ligase, which characteristically targets proteins for regulated protein degradation. The well-known targets of VHL, hypoxia-inducible factor (HIF)-1alpha and/or HIF-2alpha, are stabilized in VHL-deficient RCC.

Stabilized HIF-alpha subunits bind to the aryl hydrocarbon receptor nuclear translocator (ARNT). The heterodimer translocates to the nucleus and transcriptionally regulates multiple target genes. HIF-1alpha and HIF-2alpha are structurally similar but demonstrate opposing properties, where HIF-1alpha/ARNT drives transcriptional upregulation of the pro-apoptotic gene, BNip3, hypoxia-induced glycolytic genes, and induces cell cycle arrest by functionally counteracting Myc (84, 123). On the other hand, HIF-2alpha/ARNT drives transcriptional protumorigenic expression of genes, including the cell cycle protein Cyclin D1, the mitogen transforming growth factor (TGF)-alpha, and the angiogenic factor vascular endothelial growth factor (VEGF) (72, 123). Interestingly, expression of HIF-2alpha alone in RCC reduces BNip3 expression and does not mediate hypoxia-induced glycolytic gene expression (Hu 2003, Raval 2005). These data, together with additional genetic, molecular, and biochemical evidence, suggest that HIF-2alpha/ARNT drives renal carcinogenesis in VHL-deficient cells (83, 89, 123, 157, 167). The role of reactive oxygen species (ROS) in the regulation and maintenance of HIF-alpha expression in the absence of VHL or during hypoxia is discussed later. pVHL mediates other biological roles through protein–protein interactions (8, 31, 85, 116).

Papillary Carcinoma (10% of All RCC)

Papillary carcinomas are hereditary and can develop as individual or multiple tumors appearing either in the same kidney or in both kidneys. There are two types of papillary

FIG. 1. Anatomical structure of the nephron. Cartoon illustration of the anatomical segments of the kidney nephron and the histological kidney cancer subtypes in which they arise.

cancers: hereditary papillary RCC (HPRCC), type 1 and hereditary papillary and leiomyomatosis, type 2. Type 1 papillary RCC is more common than type 2, is slow growing, and is characteristically basophilic (takes up the basic hematoxylin dye). HPRCC type 1 arises from gain-of-function mutations in *c-met*, a gene that encodes for the tyrosine kinase c-Met receptor. Constitutive activation of the c-Met receptor drives cell growth and motility in epithelial cells. Type 2 papillary RCC is aggressive and characteristically eosinophilic (takes up the acidic eosin dye). Type 2 papillary tumors arise from mutations in fumarate hydratase (*FH*), a gene that gives rise to two FH gene products: one with a mitochondrial signal protein and the other lacking the mitochondrial signal. These tumor cells are characteristically arranged as (tubulo) papillary architecture and exhibit centrally located small nuclei, whereas the cytoplasm contains few organelles.

Chromophobe (4–5% of All RCC)

Chromophobe is a rare renal cancer, is less aggressive by comparison of other subtypes, and is likely derived from the distal renal tubular epithelium of the cortical collecting duct. Mutations to the Birt-Hogg-Dubé' (*BHD*) gene give rise to chromophobe RCC. Chromophobe tumors can reach a large size before there is any risk of spreading outside the kidney.

Collecting Duct (Less Than 1% of All RCC)

Renal cancers arising from the collecting duct are very rare. These cortical tumors arise from the epithelium of Bellini's ducts in the distal portion of the nephron, are very aggressive, and do not respond to drugs. The tumor cells have a characteristic basophilic cytoplasm, and nuclei are anaplastic. The tumors arise from an unknown etiology and their biology is less characterized.

Heterogeneity of Tumors (Mediators, Effectors, and ROS)

Introduction

Tumor heterogeneity creates a diversity of biological effects and responses within a single tumor and contributes to the molecular basis of metastasis and drug resistance. We suggest that tumor heterogeneity, in a solid tumor, is driven by initial intra- and secondary inter-tumor mediators through NADPH oxidase (NOX)- and mitochondrial electron transfer chain (ETC)-dependent increased and augmented reactive oxygen species (ROS) (Fig. 2).

The field of oxidative stress (a cellular pro-oxidant state) is complicated, and the roles and sources of ROS involved in investigated biological outcomes are too often simplified and over inferred. Several comprehensive reviews are available (10, 11, 121, 122, 150): (i) There are a number of enzymatic sources that produce ROS and conversely, a number of documented antioxidant mechanisms to neutralize ROS. (ii) Proteins, lipids, and nucleic acids are targeted by ROS, where the target of ROS is likely within close proximity to the source of ROS. (iii) Chiefly, basal oxidation of a protein, lipid, or deoxyribonucleic acid (DNA) is reversible. On the other hand, augmented and unregulated ROS can lead to irreversible modifications, resulting in constitutive redox-linked signaling events, genomic instability, or apoptosis in cells with intact apoptotic mechanisms.

Atomic oxygen has two unpaired electrons in its outer electron shell, making it vulnerable to ROS formation, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\cdot$), and peroxynitrate ($OONO^-$). Superoxide free radicals are dismutated to H_2O_2 by superoxide dismutase, SOD1 (cytosolic), or SOD2 (mitochondrial) and H_2O_2 is converted into water and oxygen by catalase enzymes. Although superoxide (O_2^-) is not a strong oxidant, it is a

FIG. 2. Schematic representation of major sources of ROS in renal cancer. The major sources of oxidative stress in the kidney during renal carcinogenesis, NADPH oxidases of the NOX family, isoforms NOX1 and NOX4, and the ETC of the mitochondria, complexes I and III. ETC, electron transfer chain; NOX, NADPH oxidases; ROS, reactive oxygen species.

precursor of most other ROS that propagate subsequent oxidative chain reactions. Superoxide can react with nitric oxide, forming peroxynitrate (OONO^-), and H_2O_2 can react with ferrous iron (Fe^{2+}), forming the highly reactive hydroxyl radical ($\text{OH}^\cdot$).

Two major sources of ROS, and the focus of this article, include the ETC of the mitochondria and the NADPH oxidases of the NOX family (NOX oxidases). Under physiological conditions, electrons “leak” from the mitochondrial ETC as a byproduct of cellular respiration, converting $\sim 1\text{--}3\%$ of oxygen molecules into superoxide ($\text{O}_2^\cdot$) at the levels of complex I (NADH/ubiquinone oxidoreductase) and complex III (ubiquinol/cytochrome c oxidoreductase) (22, 64). ETC-derived ROS generated during basal respiration are neutralized by antioxidants. On the other hand, NOX-derived ROS are produced in response to growth factors, hormones, cytokines, and other cellular events such as oxygen availability or differentiation. NOX-derived ROS predominantly serve as intra- and intercellular messengers through oxidation of susceptible cysteine residue(s) within an adjacent or proximal protein. This direct/indirect event will lead to altered biological outcomes.

In cancer cells, direct changes in expression of ETC- or NOX oxidase subunits can alter the ROS produced by the independent sources. Alternatively, indirect changes in cellular respiration, as occurs through the Warburg effect, can also contribute to alterations in ROS produced by the independent sources. Subcellular compartmentalization of ROS and the consequences on ROS targets will alter the cellular state, positively and/or negatively. The contribution of the combination of ETC- and NOX-derived ROS to cell apoptosis remains obscure and is likely variable during carcinogenesis.

We postulate that tumor heterogeneity begins early in tumorigenesis, starting with the first genetic hit that gives rise to the start of the carcinogenic process. As these genetically altered cells rapidly grow and escape cell death, they will be exposed to initial intra-tumor mediators such as variations in oxygen and nutrient availability and secretion of hypoxia-induced inflammatory cytokines. These intra-tumor mediators will increase ROS, creating an enhanced primary oxidative stress environment. Increased ROS will mediate changes in protein and lipid oxidation, giving rise to altered gene expression and activation/inactivation of signaling pathways.

A second layer of inter-tumor factors driving the evolution of tumor heterogeneity, including mediators generated from comorbidities, such as metabolic syndrome (diabetes, hypertension, obesity, and inflammation), must be considered. We postulate that these secondary events amplify NOX and likely other putative minor sources of ROS, thus augmenting oxidative stress within the tumor environment. Unleashed ROS can modify DNA, resulting in additional genetic mutations and/or changes in gene expression (Fig. 3).

NOX Oxidases as Primary and Secondary Sources of Oxidative Stress During Tumor Heterogeneity Evolution: Role of NOX1 and NOX4

NADPH oxidases of the NOX family are a major source of ROS in renal cancer and are involved in the initiation and progression of renal carcinogenesis (10, 11). Seven membrane-bound NOX catalytic isoforms, referred to as NOX1 to NOX5, dual oxidase 1 (DUOX1), and DUOX2, have been identified (6, 7, 29, 41, 49, 50, 86, 134, 139). When activated, NOX oxidase complexes take electrons from NADPH, passing them through membranes *via* the flavin adenine dinucleotide (FAD) and haem binding centers of the NOX catalytic subunit to molecular oxygen. One-electron reduction of molecular oxygen (O_2) generates superoxide anion ($\text{O}_2^\cdot$), which is then dismutated to H_2O_2 , either spontaneously at an acidic pH or catalytically by SOD. The NOX isoform, NOX4 was cloned from the kidney and is the most abundant NOX isoform expressed in renal proximal tubular epithelial cells, followed by NOX1 (50, 134). NOX4 expression has been detected in proximal tubular epithelial cells, renal mesangial cells, and the mouse collecting duct cell line, mpkCCDcl4 (13, 43, 54). Expression of NOX catalytic and regulatory subunits in the collecting duct and chromophore renal cancer has not been defined. Additionally, a role for NOX-derived ROS in HPRCC, type I is unknown. In normal hepatocytes, prolonged exposure of hepatocyte growth factor (HGF) stimulation of HGF/c-Met signaling reduces NOX4, p67phox, and p22phox expression, which is concomitant with increased viability (34). It is unclear whether the aforementioned NOX subunits are down-regulated in HPRCC, type I, and if so, whether this is a mechanism by which type I HPRCC cells maintain cell

FIG. 3. Schematic model of intra- and inter-tumor mediators, ROS, and biological effects. Variations in nutrients, oxygen, and inflammation-induced cytokines (intra-tumor mediators) activate NOX- and ETC-derived ROS. Metabolic syndrome (diabetes obesity, hypertension, and inflammation) contributes to inter-tumor mediators that amplify NOX- and ETC-derived ROS. Increased and amplified ROS result in protein, lipid, and DNA oxidation to drive tumor evolution. DNA, deoxyribonucleic acid.

viability. In FH-deficient HPLRCC type 2, p47phox-dependent NOX oxidase stabilizes HIF-1 α (138), suggesting a role for NOX1 complexes.

Variations between NOX1 and NOX4 subcellular distribution and agonist-driven activation suggest spatiotemporal regulation of NOX targets. NOX1 localizes predominantly to the plasma membrane and has been detected at lipid rafts (30). Activation of NOX1 requires translocation of the regulatory cytosolic subunits NOX organizer-1 (NOXO1), a p47phox homolog; NOX activator-1 (NOXA1), a p67phox homolog; and Rac1 (7, 30, 49, 87, 130). On the other hand, unlike NOX1, NOX4 does not require cytosolic subunits for activation. Although p22phox, a small membrane-bound protein that heterodimerizes with a subset of NOX catalytic subunits, is non-dispensable for activation of NOX1, an obligatory role for p22phox-dependent NOX4 activation is likely agonist and cell-type specific and subcellular localization dependent. NOX4 has been shown to be associated with intracellular membranes of compartments or organelles such as focal adhesions, endoplasmic reticulum, plasma membrane, nucleus, and mitochondria (7, 12, 11, 87). We were the first to show that NOX4 harbors a mitochondrial targeting sequence and localizes to the mitochondria in renal proximal tubular epithelial cells (12). This finding demonstrates an independent source of ROS within the mitochondria and suggests that novel crosstalk may exist between NOX4 and the mitochondria ETC in renal cancer, an active area of investigation in our laboratory.

The ETC of the Mitochondria as Primary Source of Oxidative Stress During Tumor Heterogeneity Evolution

Mitochondria are a critical site of ROS production in cells and tissues. It is important to note, as discussed earlier, that the NOX isoform, NOX4, localizes to the mitochondria and contributes to ROS generated within this compartment. Therefore, the ROS detected within the mitochondria can be derived from NOX4 (NOX4-derived ROS within the mitochondria) or from the ETC (complexes I and III) of the mitochondria during cellular respiration (ETC-derived ROS). The topology and regulation of NOX4-derived ROS within this organelle and their yin-yang influences with ETC-derived ROS have not been characterized. Meticulous analysis shows that complex I (NADH/ubiquinone oxidoreductase) of the mitochondrial ETC generates superoxide toward the mitochondrial matrix, whereas complex III (ubiquinol/cytochrome c oxidoreductase) generates superoxide toward the cytosol and the mitochondrial matrix (106). Importantly, superoxide can leak to the cytosol from the mitochondrial matrix through the Voltage-dependent anion channels (92). Alternatively, the superoxide within the matrix is converted to H₂O₂ by manganese-dependent SOD (MnSOD or SOD2), which freely diffuses through membranes.

In normal cells, mitochondria break down nutrients to produce energy in the form of adenosine triphosphate (ATP), a process known as oxidative phosphorylation (OXPHOS). However, cancer cells are commonly in an altered metabolic state in which ATP production occurs predominately in the cytoplasm, a process known as the Warburg effect or metabolic reprogramming (152, 153). Altered metabolism is mediated by various intracellular changes, *i.e.*, alterations of

gene expression and pathway flow that divert metabolites away from the mitochondria to support growth and viability as well as environmental cues (hypoxia and nutrient availability). Importantly, during carcinogenesis, altered metabolism will couple to chemoresistance. We postulate that enhanced, un-neutralized mitochondrial ETC-derived ROS *via* complexes I and III participate early in renal carcinogenesis when glucose uptake is heightened during active OXPHOS but not in cells that undergo the metabolic switch (22, 64).

Mitochondrial dysfunction, enhanced OXPHOS-driven metabolism, or genetic alterations of mitochondrial DNA are potential mechanisms by which ETC-dependent ROS generation is enhanced in cancer cells. Tumor cells shown to exhibit mitochondrial dysfunction are those that have mutations in the tricarboxylic acid cycle enzymes such as succinate dehydrogenase (*SDH*) or FH. Mitochondrial dysfunction in renal oncocytomas (*BHD*) is linked to mutations in subunits of complex I (100). Electron microscopy of renal tumors has also demonstrated changes in the number, shape, and function of mitochondria (145). Chromophobe renal carcinoma exhibit abnormal mitochondria with altered cristae, suggesting compromised mitochondrial function (104). However, in general, rigid studies suggest that mitochondria are not dysfunctional; rather, the diversion of metabolites away from the mitochondria, due to metabolic reprogramming, simply reduces mitochondrial activity. Therefore, we postulate that the metabolic shift (Warburg effect) reduces the role of ETC-derived ROS.

Therapeutic targeting of the enzymes that produce or neutralize ROS as a strategy for cancer treatment is a highly contentious area of study due to the variability of results generated between controlled *in vitro* experiments and *in vivo* animal models. Moreover, validation of the results, as analyzed through clinical studies, is largely inconclusive. Together, this suggests that tumor heterogeneity and comorbidities are causal factors and therapeutic efficacy will likely be enhanced through combinational targeting and identification of windows of opportunity during carcinogenesis (11, 16, 48, 161).

Intra-tumor Mediators and Renal Cancer: Role of Enhanced NOX- and ETC-Derived ROS

Tumor suppressor loss of function, alterations in oxygen, and/or nutrient availability result in NOX and ETC production of ROS and intra-tumor heterogeneity.

NOX4 and p22phox are upregulated in cultured VHL-deficient cells and in human RCC tissues, concomitant with enhanced NOX-derived ROS (13, 14). Importantly, NOX-derived ROS is reduced on VHL reintroduction (13). On the other hand, reintroduction of VHL to VHL-deficient cells enhances intracellular ROS, as measured by the broad non-specific dyes, dichlorofluorescein and dihydroethidium. Enhanced cytosolic detection of ROS described by reintroduction of VHL is likely mediated by restoration of ETC-derived ROS, as mitochondrial biogenesis and oxygen consumption levels are improved (60). VHL-deficient cells express HIF-2 α alone or HIF-1 α /HIF-2 α simultaneously. Hypoxia-induced, ROS-dependent stabilization of HIF-1 α is an acute event. Both NOX- and mitochondrial-derived ROS have been implicated in hypoxia-induced stabilization of HIF-1 α expression through transcriptional

and post-translational mechanisms (20, 56, 60, 142). On the other hand, NOX-derived ROS maintain expression of HIF-2 α in VHL-deficient cells through mammalian target of rapamycin C2 (mTORC2)-dependent translational mechanisms (111), suggesting more chronic events.

Solid tumors exhibit intratumor hypoxic states, where regions of low oxygen (hypoxia) and necrosis are common (99). Accumulating evidence suggests that NOX oxidases, NOX1 and NOX4, serve as oxygen sensors. The human NOX4 promoter harbors hypoxia-responsive elements, which bind HIF-1 (38). Similarly, NOX1 mRNA and protein expression are enhanced in lung cells that are exposed to hypoxia (56). Hypoxia-induced activation of NOX1-dependent ROS production is necessary for activation of HIF-1-dependent gene expression that is blocked by the antioxidant catalase (56). In support of these conclusions, NOX1 and NOX4 are increased in mice that are exposed to chronic hypoxia (102). Silencing or inhibition of NOX1, but not NOX4, inhibits NF- κ B (NF- κ B)-dependent endothelial cell migration *in vitro* and impairs angiogenesis through peroxisome proliferator-activated receptor- α (PPAR α) *in vivo* (48, 102). Interestingly, VHL-deficient cells that are exposed to hypoxia induce interleukin (IL)-6 and IL-8 production in an NOX4-dependent manner (44). The significance of this is that IL-6 stimulates cell migration and invasion in VHL-deficient cells (44). Notably, neither normal proximal tubular epithelial cells that are exposed to hypoxia nor VHL-deficient RCC cells that are exposed to normoxia secrete IL-6 or IL-8 (44). TGF- β is a pleiotropic cytokine exerting positive and negative biological outcomes (cell proliferation, epithelial-mesenchymal transition (EMT), migration, invasion, and drug resistance) during carcinogenesis, and this is known as the TGF- β paradox (154). This may be due to TGF- β 's role in tumor heterogeneity. Interestingly, Oshimori *et al.* have dissected variations in biological outcomes between TGF- β -mediated responding- and non-responding cancer stem cells, suggesting non-genetic paradigms (115). Non-responding TGF- β squamous cell carcinoma stem cells accelerate tumor growth, whereas responding TGF- β squamous cell carcinoma stem cells show transcriptional activation of p21-dependent nuclear respiratory factor 2 (NRF2)-driven antioxidant properties and drug resistance (115). Stem cell analysis in human renal carcinoma tissue indicates two major populations CD133⁻/CD105⁺/CD24⁻ and CD133⁺/CD105⁻/CD24⁺ (21, 46). The molecular interplays between NOX4 and TGF- β are well established (18, 35, 69, 73, 136–164, 166). As CD105 is a receptor for TGF- β , it would be interesting to determine whether pleiotropic effects exist in RCC stem cells and whether NOX4-derived ROS play a biological role (78). Independent studies suggest that TGF- β and HIF share an overlap of gene regulation, where TGF- β inhibition attenuates the invasive capacity of ccRCC cells (7, 17).

The role of NOX4 in the regulation of cytoplasmic/nuclear protein trafficking of key transcription factors is evident; however, the mechanisms remain unknown. NOX4 silencing in cardiomyocytes excludes NRF2 from the nucleus, suggesting that NOX4 paradoxically regulates key antioxidant pathways (19). This observation was extended to renal cancer, where silencing of NOX4 excludes HIF-2 α from the nucleus and blocked cell branching, colony formation, and growth in a Xenograft model (58). p53, a key transcriptional

regulator of damage- and stress-induced cell death, is often mutated in cancer. However, in renal carcinoma, the p53 pathway is commonly intact. The redox regulation of p53 is both direct and indirect and notably results in activation or inhibition of p53 transactivation properties (27, 140, 148, 149). Oxidation of p53 has been described. Oxidation of noted cysteine residues within the p53 DNA binding domains leads to the inhibition of p53 DNA binding (140, 149), whereas cis-diamminedichloroplatinum II -induced oxidative stress prompts p53-dependent p21 transactivation (148). This suggests that p53 cysteine oxidation and translational activation may serve as biomarkers of cancer prognosis or therapeutic efficacy, as suggested (16). In support of this, Imipramine blue inhibits NADPH oxidase-dependent activation of NF- κ B-induced EMT/cell invasion in glioma cells and head and neck cancer (109, 161).

The role of ETC-derived ROS in primary angiogenesis, cytokine production, and invasion in RCC remain less characterized. In endothelial cells that are exposed to hypoxic conditions, superoxide is formed at the ubisemiquinone site of complex III in the mitochondria (26, 135); however, in normal cells that are exposed to hypoxia, OXPHOS-dependent ATP is reduced and depending on the duration of hypoxia, the cell will adapt by inducing glycolysis or undergoing cell death. Hypoxia, therefore, mimics a Warburg-like effect.

Inter-tumor Mediators (Metabolic Syndrome) and Renal Cancer: Role of NOX- and ETC-Derived Amplified ROS

Metabolic syndrome is on the rise worldwide. Metabolic syndrome is a broad umbrella term that encompasses a number of chronic disease-associated risk factors, including high blood sugar (diabetes), blood pressure (hypertension), triglycerides, and abdominal fat (obesity), all of which contribute to systemic chronic inflammation. Epidemiological evidence suggests that metabolic syndrome is associated with increased risk of cancers at multi-sites, but it may be gender and age related (120, 127). Unlike epidemiological approaches to determine the increased risk of developing renal cancer in those who have metabolic syndrome, we will discuss the putative contribution of metabolic syndrome in the underlying development of renal cancer. This is an understudied area of investigation, likely due to the lack of genetic animal models. The contribution of chronic diseases defined under metabolic syndrome (high blood pressure, type-2 diabetes, obesity, and inflammation) as secondary mediators of amplified NOX-derived ROS and tumor heterogeneity is evident. Notably, obesity is a risk factor for type-2 diabetes and hypertension, and there is a link between obesity, diabetes, and hypertension. Furthermore, both obesity and diabetes contribute to an underlying low-grade inflammatory state. Literature describing the molecular connection between hypertension and cancer are lacking; therefore, we will focus the discussion toward obesity, diabetes, and renal cancer, with the inclusion of inflammation within these topics.

Obesity and Renal Cancer

Obesity is defined as excess adipose tissue. Adipose tissue can be subcutaneous (resides between the skin and muscle)

or visceral (resides within the main cavities of the body). Visceral adipocytes release free fatty acids (FFA) into the blood that are carried coupled to albumin. The renal proximal tubule retrieves albumin-bound FFA from the filtrate by receptor-mediated albumin endocytosis (33, 103). As previously mentioned, conventional renal carcinoma has a clear cell characteristic phenotype due to deregulated intracellular lipid biosynthesis that is contributed by sterol regulatory element-binding proteins (SREBP) gene regulation and mTOR signaling in these tumors (39). The effects of extracellular FFA uptake by VHL-deficient proximal tubular cells and adjacent uninvolved tissue during obesity-associated renal carcinoma is unknown, although they may contribute as a stored energy source to support rapid cell growth and cell division. Extracellular and intracellular contributions of the key inflammatory pathways that are upregulated by lipids include NF- κ B and signal transducer and activator of transcription (STAT)-3, leading to transcription of genes that mediate cell survival and metastasis (2). NF- κ B and STAT-3 are well-characterized signaling pathways that are activated in RCC, and their activity is sustained by NOX-derived ROS. Additionally, NOX-derived ROS regulate mTORC2 activity in clear cell carcinoma (111); however, the roles and mechanisms by which NOX oxidases participate in lipid biosynthetic pathways through mTORC2 need further investigation.

Obesity is a strong inducer of OXPHOS gene expression and activity. An examination of OXPHOS gene expression indicates that it is higher in the livers of diabetic individuals compared with controls (144). Beta-oxidation of FFA in the mitochondria generates acetyl-CoA, which enters the citric acid cycle. Thus, utilization of FFA as an energy source is a mechanism contributing to ETC-derived ROS in the obese environment. Importantly, multivariable-adjusted hazard ratio studies show that use of statins, cholesterol-reducing drugs, in patients with cancer is associated with reduced cancer-related mortality (113). PPARs are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily. PPAR α is mainly expressed in the liver, where it activates fatty acid catabolism. PPAR α activators have been used to treat dyslipidemia, causing a reduction in plasma triglyceride and an elevation of high-density lipoprotein cholesterol. More research is needed to determine the effects of statins on intracellular lipid biosynthesis in VHL-deficient cells and on cell growth, metastasis, and drug resistance.

Excess pro-inflammatory adipokines that are generated and released into the obesity milieu will give rise to a systemic chronic inflammation state. Exogenous inflammatory cytokines can induce NOX4-derived ROS, cell migration, and metastasis in VHL-deficient renal cancer cells (44). Interestingly, the obesity paradox in renal cancer describes an inverse relationship between body mass index (BMI) and survival in advanced-stage RCC (24, 76). The mechanisms behind this finding are currently unknown. A comprehensive genomic and molecular analysis (genomic instability, DNA methylation, and gene expression) of 126 patients with high BMI and RCC did not reveal specific DNA mutations, increased instability, or DNA hypermethylation. However, the investigators collectively identified statistically significant differences in gene expression of metabolic genes and conclude that the tumors developing in obese patients may be due to indolence compared with normal-weight patients (61, 62).

Diabetes and Renal Cancer

The global prevalence of type-2 diabetes is on the rise. However, the links and underlying associated mechanisms between diabetes and renal cancer remain unclear. Type-2 diabetes is a state of insulin resistance resulting in high levels of fasting blood glucose levels, >126 mg/dL. Insulin resistance also leads to chronic elevation of circulating insulin and insulin-like growth factor-1 (IGF-1) in the blood. Renal proximal tubular and tubular duct cells express insulin receptors and are sensitive to insulin levels (63, 97). Further, the IGF signaling axis is dysregulated in clear cell renal cancer but not evident in papillary or chromophobe (143). Activation of the insulin/IGF signaling axis mediates cell proliferation and survival (136). IGF-1 stimulates NOX4 expression and activation in renal proximal tubular cells (52, 112). However, the effects of IGF-1 on NOX4-mediated cell survival in RCC remain unknown. Thiazolidinediones (TZDs) are insulin sensitizers that act through PPAR γ and are used to treat type-2 diabetes. Similar to statins, evidence suggests that PPAR modulators may have beneficial effects as chemopreventative agents in carcinogenesis (131, 141). Alpha-linolenic acid, an omega fatty acid, inhibits cell proliferation in cultured clear cell OS-RCC cells by activating PPAR γ (159). The glucose receptor (GLUT-1) is highly expressed in VHL-deficient RCC due to stabilization of the master transcriptional regulators, HIFs. High levels of glucose in the diabetic milieu enter the cell through GLUT-1. Glucose activates NOX4 within the mitochondrial compartment in normal renal cells (12). The effects of glucose-induced NOX4 in renal cancer are currently unknown.

High-Throughput Analysis and Renal Cancer

High-throughput approaches have been utilized for the discovery of novel prognostic and diagnostic biomarkers to define cancer subtypes in renal cancer and/or as potential therapeutic targets. Later, we will discuss the molecular crosstalks between NOX- and ETC-derived ROS and the regulation of miRNA, transcriptomics, metabolomics, and epigenetics (Fig. 4). We will also reference, where applicable, comprehensive reviews outlining the results obtained from these high-throughput approaches in renal cancer. The analysis of complex and comprehensive systems requires bioinformatics and systems biology tools. At this time, a substantial amount of data have been generated through consortium efforts supported by the National Institutes of Health (NIH) and collaborative institutions in tissue collection, experimental analysis, and data interpretation, with subsequent releasing of the results to the scientific community, which provides an enriched amount of information for translational research in kidney cancer.

The Cancer Genome Atlas (TCGA), started in 2005 by the NIH and participating institutions, was launched to catalog genetic mutations that are responsible for cancer using genome sequencing and bioinformatics. This consortium collected thousands of cancer types (more than 33) and executed several high-throughput analyses, including gene expression and copy number variation profiling, single nucleotide polymorphism genotyping, DNA methylation landscape, miRNA profiling, and exon sequencing. The annotated bioinformatics results are publically available for data

FIG. 4. High-throughput screening approaches and renal carcinogenesis. Single and integrated analysis using high-throughput approaches to evaluate epigenetic modifications (DNA methylation), micro-RNA expression and function in the cytosol and mitochondria (oncoMIR and mitoMIR, respectively), metabolites (oncometabolites), and transcriptomics and their interplays with NOX- and mitochondrial ETC-driven oxidative stress in renal cancer. RNA, ribonucleic acid.

mining through the TCGA data portal (<https://tcga-data.nci.nih.gov/tcga>). Comprehensive molecular characterization of 400 clear cell carcinoma cancer tissues by the Cancer Genome Atlas Research Network study identified a number of mutated genes corresponding to widespread pathway activation and DNA hypomethylation (23). On the other hand, Chromophobe RCC displayed a low rate of mutation and distinct metabolic profiles (36). Data mining by independent researchers around the globe with various background expertise has accessed the large data analysis and expanded the research using molecular, biochemical, mathematical, and animal model approaches to predict metastasis, biomarkers, and prognostic and clinical outcomes, resulting in more than 77 publications to date.

TCGA data mining and mutational analysis of specific NOX isoforms and their protein partners have not been published. In part, this is due to the lack or limited available information on NOX isoforms within these large datasets. However, aberrant expression and regulation of NOX-dependent redox targets have been described and are discussed later. Moving forward, comprehensive and integrative analysis between major ROS producing- and ROS-consuming systems may highlight novel redox-regulated landscapes.

Epigenetic Modification in Renal Carcinoma

Epigenetics is defined as inherited, dynamic, and reversible modifications in gene expression that are not encoded in the DNA sequence (114). DNA methylation plays an important role in carcinogenesis, where hypermethylation of CpG islands within the promoter of tumor suppressor genes or hypomethylation of oncogenes will alter expression and functional landscapes within tumors (42). In mammalian cells, *de novo* DNA methylation is an enzymatic modification by which a methyl group is added to cytosine by DNA methyltransferases (3). Demethylation is a process that allows epigenetic reprogramming and is important for progression of tumor development. The ten-eleven translocation (TET)

family of 5-methylcytosine hydroxylases promotes demethylation through sequential oxidation modification of the cytosine bases. Oxidative metabolism regulates DNA methylation events through the TET family of dioxygenases (101), suggesting that the metabolic switch may alter the epigenetic landscape in renal cancer. Indeed, metabolic disruption alters the production and availability of cofactors that are needed for epigenetic regulation (70). Recently, Shim *et al.* demonstrated high levels of the L-enantiomer of 2-hydroxyglutarate (L-2HG), an inhibitor of TET enzymes, concomitant with reduced levels of 5-hydroxymethylcytosine in human RCC compared with the normal control (133). This study highlights novel mechanisms regulating the epigenetic landscape in RCC.

Several genes that are hypermethylated in RCC have been described, including the *VHL* (tumor suppressor) (67), *Basonuclin (BNC1)-1*, Collagen type XIV (*COL14A1*), secreted frizzled-related protein 1 (*SFRP1*) associated with poor prognosis and *BNC1*, Cystatin E/M (*CST6*), Reprimo (*RPRM*), *SFRP1* associated with cell growth (105, 125), glutathione peroxidase 3 (*GPX3*), a scavenger of ROS (91), Wnt inhibitory factor-1 (*WIF-1*), a tumor suppressor and an inhibitor of WNT signaling (77), and *Rap1GAP*, a negative regulator of Rap and a driver of cell invasion (80). The role of NOX oxidases or ETC-derived ROS as an upstream direct or indirect regulator of DNA methylation or as a downstream target of methylation is unknown and needs investigation. Other recent reviews outlining large datasets evaluating DNA methylation as a regulator of the epigenetic landscape are listed (65, 82, 132). Epigenetic-driven gene expression profiles are dynamic, and, therefore, the complexity added due to obesity (59) and diabetes (126) needs to be evaluated systematically.

miRNA and Renal Cancer

miRNAs are small (22 nucleotides), non-coding RNA molecules that post-transcriptionally regulate gene expression through base pairing with complementary sequences within

mRNA molecules. Deregulated miRNAs that are associated with cancer have been coined “oncomirs.” A typical miRNA can regulate more than 400 conserved targets and repress the production of hundreds of proteins that mediate biological processes, including cell growth, proliferation, EMT, migration, invasion, drug resistance, markers of prognosis, or RCC subtype classification (15, 94, 165). An miRNA Cancer Association database is a collection of miRNA expression profiles in various cancers available to the research community (mircancer.ecu.edu). A number of studies examining miR expression profiling and their effects on prognosis, classification of RCC subtype, signaling pathways, or biological outcomes in cultured RCC cells and/or human RCC tissue from clear cell, HPRCC, and chromophobe have been documented and reviewed (1, 25, 28, 32, 37, 55, 57, 66, 74, 81, 88, 90, 93, 107, 108, 110, 117, 124, 137, 155, 156, 162).

Studies examining the upstream and downstream regulation of miRNAs on oxidative stress in human disease are recently becoming available. In endothelial cells, mechanical forces (shear stress)-regulated endothelial responses were used to identify miRNAs that mediate oxidative stress and inflammation. This comprehensive study, in part, found miR-25 and -23b as upstream negative regulators of NOX4, MiR-17-5p, and -20a, and Let-7G as an upstream regulator of p47phox (an NOX1 binding partner) (45, 98). On the other hand, miR-146a inhibits NOX4 in endothelial cells in response to high glucose (151). In endothelial tumors, miRNA-21a-3p targets NOX4 to inhibit tumor growth (53). In lung carcinoma, miR-21 inhibits NOX4-driven lung metastasis (158). miRNAs have also been well studied in the regulation of mitochondrial metabolism in carcinogenesis, and they are reviewed (9, 146). MitomiRs are mitochondrial miRNAs that target mtDNA (5, 40). MitomiRs may have intimate roles in the regulation of OXPHOS function and cell viability. miRNAs that target mitochondrial genes are involved in the regulation of pro-oxidant (miR-335, -34a, -338, -210, and -181c) or antioxidant (miR-145, -23b, -210) effects (4). These studies highlight the variations between miR expression in different cell types and environments. It would be interesting to document miR variations during loss of genes that give rise to renal carcinoma, such as VHL, c-MET, FH, SDH, and BHD. How the miRs mentioned earlier and their targeted expression are additionally altered during metabolic syndrome remains to be determined; however, the roles of miRs in human diseases are reviewed: miRNA and cardiac regeneration (71), miRNA and obesity-associated disorders (1), miRNAs in kidney physiology and disease (147), and oxidative stress and miRNAs in vascular disease (95).

Metabolomics

Metabolomics is defined as the non-targeted analysis of all small-molecule metabolites produced by the body. Using metabolomics as an approach helps one gain a broad picture between normal physiology and pathophysiology. In cancer, detected metabolites are called “oncometabolites.” Oncometabolites are generated and accumulated during the metabolic reprogramming (Warburg effect). As such, the studies highlighting aberrant accumulation of oncometabolites have been generated from HPRCC or subtypes of RCC in which mitochondrial enzymes, such as FH or SDH, are dysfunctional. These studies led to the discovery that HIF-

1 α is stabilized by succinate and fumarate metabolites that accumulate during the loss of *FH* and *SDH* genes, respectively (119). This finding linked metabolomics to transcriptomics, as HIF-1 α is a key transcriptional regulator during hypoxia and metabolic reprogramming. Metabolomics and DNA methylation approaches led to the recent discovery of a new potential oncometabolite in clear cell renal cancer, L-2HG, which arises from a reduced copy number of the gene L-2-hydroxyglutarate dehydrogenase (*L2HGDH*) (128, 133).

Metabolites are stable and found in urine. Measurement of metabolites in urine and other biofluids is a strong approach of viewing pathophysiological profiles in renal cancer, diabetes, obesity, and inflammation due to the non-invasive methods of sample collection. Urine analysis in individuals with RCC identified quinolinate, 4-hydroxybenzoate, and gentisate, which are involved in pathways of amino acid and energetic metabolism, consistent with cancers exhibiting a Warburg phenotype (79). The detection of acylcarnitines in the urine during a separate study suggested a biomarker of advanced RCC (47). The role of NOX oxidases, upstream or downstream, of cancer metabolism has not been studied, but it is an active area of investigation in our laboratory. As this field progresses, it will be of clinical interest to combine the large datasets from intracellular glycolytic- and Warburg-derived oncometabolites with systemic metabolites derived from metabolic syndromes.

Transcriptomics

Gene expression microarrays and RNA-seq are current approaches to evaluate transcriptome data in a collected data sample or at the single-cell level. Microarrays were developed in the 1990s, can differentiate expression between cell and tissue types, and illustrate expression changes across diseases such as cancer. However, limitations of microarrays include background hybridization, limited accuracy of expression for transcripts in low abundance, and inability to detect splice variants or unknown genes. RNA-seq is a high-throughput approach generating orders of magnitude of data compared with microarrays and, thus, generally requires high-level informatics to extract meaningful results. RNA-seq can detect splice variants, isoforms, and new genes. Investigators are taking advantage of large datasets of the RNA-seq and meta-data of kidney renal clear cell carcinoma (KIRC) from the TCGA data portal. Analysis of gene profiling between RCC subtypes showed distinctive gene expression patterns among them (68). These early gene expression studies have played a large role in our understanding of the biology of RCC, as many key regulators of renal tumorigenesis are mediated by master transcriptional factors such as HIF-1 and HIF-2 α , NF- κ B, beta-catenin, and NRF. Notably, all the aforementioned transcription factors are redox sensitive, and their activity is regulated by NOX-derived ROS. With the availability of instrumentation and expertise, cancer researchers are combining transcriptomics with epigenetics to gain new insights into the regulation of gene expression (called integrated analysis). This is seen by Sato *et al.*, who performed an integrated analysis of more than 100 clear cell RCC specimens in whole-genome and -exome RNA-seq in parallel with microarray and DNA methylation analysis, genomic copy number, and immunohistochemistry.

FIG. 5. Tumor evolution and participation of sources of oxidative stress. Timeline of renal cancer growth from initial hit to large tumor. The major sources of ROS participating in oxidative stress during the initial hit and development to small tumor include the NOX isoforms NOX-1 and NOX-4, in addition to the OXPHOS-mitochondrial ETC. Subsequent to metabolic reprogramming (aerobic glycolysis), the mitochondria will experience diminished activity and the NOX-derived ROS will be the main contributor to oxidative stress. OXPHOS, oxidative phosphorylation.

This multi-institutional, comprehensive study identified new mutated genes and pathways involved in renal carcinogenesis (129). Unique correlations between DNA methylation, gene expression, and copy number were described (129). Malouf *et al.* used KIRC RNA-seq to identify long non-coding RNA (lncRNA) in renal cancer, which identified four-cluster lncRNA subclasses with distinct clinicopathological and

genomic features (96). Another KIRC TCGA dataset analysis to identify new significant genes and pathways in RCC was performed by Yang *et al.* Based on their hierarchical clustering analysis of disrupted pathways with associated genes involved in RCC development, the team engineered a vector machine-based supervised-learning classifier to easily identify, classify, and predict unknown samples (160). This new

FIG. 6. The effects of nutrients, oxygen, and ROS on tumor heterogeneity. As tumors grow, inner cells of a tumor will experience reduced levels of nutrients (light green color) and hypoxia (light blue color). During this time, increased ROS will drive aberrant gene expression profiles and protein function. Non-reversible changes that remain after angiogenesis restores nutrient (dark green) and oxygen availability (purple) will add to tumor heterogeneity compared with cells that had normal/excess availability to nutrients and oxygen (orange). Systemic (inter-tumor) mediators will amplify ROS and additionally add tumor heterogeneity to cells within the tumor environment (pink). Together, the heterogeneous cell population will exhibit different phenotypes toward metastasis and drug resistance.

algorithm has potential to merge and generate new clusters between the biospheres of transcriptomics and data of intra- and inter-tumor heterogeneity.

Concluding Remarks

Clinical analyses of large renal tumors reveal substantial heterogeneity within the tumors themselves, which are composed of low- and high-grade tumor regions with areas of necrosis and vascularity. This heterogeneity likely explains drug resistance, despite targeted therapies. ROS play a major role in the development and progression of renal carcinogenesis. However, all ROS should not be considered the same. We postulate that subcellular distribution of major sources of ROS and their regulation during the carcinogenic timeline will orchestrate step-by-step movement forward, starting with the first hit. Inactivation of tumor suppressor genes or activation of oncogenes in renal cancer upregulates and activates NOX-derived ROS. Stabilization of HIFs due to the inactivation or loss of *VHL*, *FH*, and *SDH* genes or reduced oxygen exposure (hypoxia) will drive upregulation of glucose receptors, allowing rapid uptake of glucose to feed enhanced glycolysis through OXPHOS and, thus, augmenting ETC-derived ROS. Therefore, with some exceptions, we propose that during initiation stages of dysplasia, both NOX-derived ROS and ETC-derived ROS contribute to increased ROS. However, when the tumor undergoes metabolic reprogramming (Warburg effect) and shunts pyruvate toward biosynthetic intermediates to support rapid cell growth and survival, we suggest that the role of the ETC-derived ROS from the mitochondria is significantly reduced due to its diminished activity, not dysfunction. Subsequent to the metabolic shift, we suggest that the NOX isoforms, NOX1 and NOX4, are the main source of oxidative stress to maintain critical steps that are involved in tumor progression, metastasis, and drug resistance (Fig. 5) (10, 11).

The contribution of intra- and inter-tumor mediators during renal carcinogenesis is evident during tumor evolution. From the time a cell undergoes the initial hit to rapid growth (<7 cm in size), the inner cells of the tumor will experience reduced nutrient and oxygen availability. We postulate that the cells will respond by activating/inactivating gene expression profiles. When the HIFs are stabilized and upregulate VEGF, angiogenesis will restore nutrients and oxygen to the environment. However, we postulate that intracellular non-reversible alterations in gene expression and protein chemistry during the primary oxidative stress state remain and the pre-exposed cells will evolve a diverse expression, biochemical, and signaling landscape profile. Inter-tumor mediators that are contributed by metabolic syndrome diseases, including diabetes, obesity, and hypertension, all of which result in an underlying low chronic inflammatory state, will drive additional changes in gene expression and post-translational modifications. Amplified ROS may additionally result in additional gene modifications/mutations (Fig. 6). Together, high-throughput data collection and analysis need to be systematically evaluated and carefully presented. As much information pertaining to known intra- (hypoxia, nutrient status, and inflammation) and inter- (comorbidities) mediators should be discussed.

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Abbreviations Used

ARNT = aryl hydrocarbon receptor nuclear translocator
 ATP = adenosine triphosphate
 BHD = Birt-Hogg-Dube'
 BMI = body mass index
 BNC1 = Basonuclin
 COL14A1 = collagen type XIV
 CST6 = cystatin E/M
 DNA = deoxyribonucleic acid
 DUOX = dual oxidase
 EMT = epithelial-mesenchymal transition
 ETC = electron transfer chain
 FAD = flavin adenine dinucleotide
 FFA = free fatty acids
 FH = fumarate hydratase
 GLUT-1 = glucose receptor

GPX3 = glutathione peroxidase 3
 H₂O₂ = hydrogen peroxide
 HGF = hepatocyte growth factor
 HIF = hypoxia-inducible factor
 HPRCC = hereditary papillary renal cell carcinoma
 IGF-1 = insulin-like growth factor-1
 IL = interleukin
 lncRNA = long non-coding ribonucleic acid
 KIRC = kidney renal clear cell carcinoma
 L-2HG = L2-hydroxyglutarate
 miRNA = microRNAs
 mTOR = mammalian target of rapamycin
 NF- κ B = NF-kappaB
 NIH = National Institutes of Health
 NOX = NADPH oxidases
 NOXA1 = NOX activator-1
 NOXO1 = NOX organizer-1
 NRF = nuclear respiratory factor
 O₂⁻ = superoxide anion
 OXPHOS = oxidative phosphorylation
 PPAR = peroxisome proliferator-activated receptors
 RCC = renal cell carcinoma
 RNA = ribonucleic acid
 ROS = reactive oxygen species
 RPRM = Reprimo
 SDH = succinate dehydrogenase
 SFRP1 = secreted frizzled-related protein 1
 SOD = superoxide dismutase
 SREBP = sterol regulatory element-binding proteins
 STAT = signal transducer and activator of transcription
 TCGA = The Cancer Genome Atlas
 TET = ten-eleven translocation
 TGF = transforming growth factor
 TZD = thiazolidinediones
 VEGF = vascular endothelial growth factor
 VHL = von Hippel-Lindau tumor suppressor gene
 WIF-1 = Wnt inhibitory factor-1