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Review Article: Xanthine Oxidoreductase-An Important Functional Component of Differentiation, Apoptosis and Aggressiveness of Tumors

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Abstract: Xanthine oxidoreductase(XOR)plays an important role in many pathologies, such as tumors, chronic hypoxia, renal injury, hypertension and so on. In this article we mainly review the current research about the regulation of activity and gene expression of XOR, and its physiochemical property, organ distribution, changes in tumors and relation with the other diseases. We conclude that XOR is an important functional component of differentiation and apoptosis and diminished expression contributes to aggressiveness of tumors.

Keywords: Xanthine Oxidoreductase, Differentiation, Apoptosis, Aggressiveness, Tumors

1. Introduction

Xanthine oxidoreductase (XOR) is a kind of molybdoflavoenzyme that catalyzes the oxidation of hypoxanthine to xanthine or xanthine to uric acid, which has two forms: Xanthine oxidase(XO) and Xanthine dehydrogenase (XDH). Under normal condition, XDH has an important effect on purine metabolism which results in uric acid, while under pathological conditions, such as inflammation, ischemic infarction, tumor and so on, oxidation of cysteine residues and/or limited proteolysis can convert XDH to xanthineoxidase(XO) [1].

The subunit of XOR is consisted of four parts: a molybdenum cofactor where purine oxidation happens, one FAD site where NAD+ and O2 reduce and two Fe/S clusters which are the conduits to transfer electron from Mo-co to the FAD [2].

Recent research reveals that the Escherichia coli periplasmic aldehyde oxidoreductase is an exceptional member of the xanthine oxidase family of molybdoenzymes. E. coli protein contains a molybdopterin-cytosine-dinucleotide cofactor and this is very common in many bacteria phyla. Researchers confirmed that the active site of PaoABC is highly exposed to the surface with no aromatic residues and an arginine (PaoC-R440) making a direct interaction with PaoC-E692, which is a catalyst in the procedure [3].

ROX has two kinds of productions: reactive oxygen species (ROS) including superoxide, hydrogen peroxide and reactive nitrogen species (RNS) including species derived from nitric oxide. These productions have the protective and deleterious effects (reviewed in [4].)

There are many relative researches and reviews are available [5-11]. In recent years, a large number of researches cover the regulation the express and activity of XOR, moreover, a great deal of new information has become available concerning relation of XOR with many diseases such as tumors, circular diseases, hypoxia pathology and so on, ranging from the genesis to the development and prognosis. This review mainly focuses on the regulation of activity and gene expression, physiochemical property, organ distribution, changes in tumors and relation with the other diseases.

2. Physicochemical Property

Conversion from XDH to XO plays a critical role in many processes including physical and pathologic processes. There are some factors which may influence the conversion, such as

temperature, PH and the C-terminal region. It was found that recombinant human XDH was incubated with subcellular fractions of human liver, a heatlabile activity was represented in the mitochondrial intermembrane space and converted XDH irreversibly to XO [12]. Under ischemia condition, xanthine dehydrogenase was converted to xanthine oxidase in all tissues with half-times of conversion at 37°C of 3.6, 6, 7, and 14 h for the liver, kidney, heart, and lung, respectively, and the time course of enzyme conversion at 40°C was greatly extended with half-conversion times of 6, 5, 5, and 6 d for the respective tissues [13]. It was found that alterations in pH between 5.5 and 7.4 did not affect the relative proportions of H_2O_2 and. O2− formation [14]. In addition to temperature and PH, the C-terminal region plays a role in the dehydrogenase to oxidase conversion. A variant of the rat liver enzyme without the carboxy-terminal amino acids 1316–1331 had 50~70% of oxidase activity even after prolonged the interaction with dithiothreitol [15].

3. Organ and Tissue Distribution

Most researchers showed that XOR mainly locates in liver and intestine (normally in duodenal). Emanon and coworkers first reported the two forms for the mouse [16]. They found that the liver form is fully converted into the duodenal form by incubation at 37°C with crude trypsin and the two forms are similar in embryo mice. Sequently, it was found that even in the intact cell the polypeptide chain of XOR is unstable and there is a very small amount of inactive XDH/XO in human liver and intestine [17]. Furthermore, using reverse transcription followed by PCR to amplify their RNA samples, the other investigators demonstrated very low levels of the XOR transcript existed in heart, brain, lung and kidney tissues [18].

It has been reported that XO/XDH exists in the cytoplasm of epithelium lining terminal ducts and majority of it is in the alveolar epithelium of lactating mammary lobules. In contrast, XO/XDH was not found in situ carcinomas and invasive carcinomas of the breast [19]. What's more, XOR has been found to release into the systemic circulation from the liver and intestine and to bind to the plasma membrane of endothelial cells via surface glycosaminoglycans during reperfusion after ischemia [20, 21].

4. Activity Regulation

There are many factors which can influence the activity of XO, including genetics and chemical materials. Several single nucleotide polymorphisms (SNP) of XO gene are involved in individual variations of XO activity. Studies showed that there are three nonsynonymous single nucleotide polymorphisms, including 514G>A (Gly172Arg), 3326A>C (Asp1109Thr), and 3662A>G (His1221Arg) in Japanese participants and a deficiency activity in two variants (Arg149Cys and Thr910Lys); low activity in six variants (Pro555Ser, Arg607Gln, Thr623Ile, Asn909Lys, Pro1150Arg, and Cys1318Tyr); and high activity (CLint: approximately two-fold higher than that in the wild-type)in two variants (Ile703Val and His1221Arg) [22].

Another research showed that using normal human chondrocytes, monosodium urate or recombinant tumor necrosis factor-α increased the activity of xanthine oxidase. However, t febuxostat, which is the xanthine oxidase-specific inhibitor, failed to inhibit this response. Activation of signal transducer and activator of transcription-3 also was seen in pellet cultures initiated from juvenile chondrocytes and MSCs incubated with MSU, recombinant tumor necrosis factor-α or febuxostat, but apoptosis was increased only in the pellet cultures derived from juvenile chondrocytes. The increased frequency of apoptotic chondrocytes in response to recombinant tumor necrosis factor-α or monosodium urate was not dependent on either activation of STAT3 or the activity of XO [23].

Activity regulations are dependent on different genetic pathways. It has been confirmed that XOR enzymatic activity is significantly increased by mechanical stress via activation of p38 mitogen-activated protein kinases (MAPK) and ERK and it plays a critical role in the pathogenesis of pulmonary edema associated with ventilator-induced lung injury (VILI) [6]. Low dose cycloheximide can induce the XOR in HC11 cells. The possible mechanism may be that low dose cycloheximide promoted the nuclear accumulation of the CCAAT/enhancer-binding protein-β(C/EBPβ) transcription factor, which activated the XOR promoter, however, the expression was blocked by inhibitors of p38 MAP kinase [24]. Microparticles (MPs) is the other activator of XOR, which stimulated ERK1/2 phosphorylation via PI3K-dependent mechanism. The function is thought to be related with NO and ROS. MPs enhanced expression of caveolin-1 and decreased its phosphorylation, what's more, they decreased NO production that was associated with overexpression and phosphorylation of endothelial NO synthase (eNOS) [25].

5. Expression and Regulation of XOR Gene and Protein

It has been found that the mutant of XOR can change the form of XOR, but also can influence the purine catabolism. It was reported that when tryptophan 335 and phenylalanine 336 were replaced with alanine and leucine respectively, it was obtained in the XO form in Sf9 cells, but parts of them would change to XDH form when incubation with dithiothreitol [26]. The other Glu803-to-valine (E803V) mutation lacked activity towards hypoxanthine completely, but had little activity towards xanthine. On the other hand, opposite results were observed in the Arg881-to-methionine (R881M) mutant [27].

Additionally, there are some substances that could regulate the expression of XOR, such as H_2O_2, calcium and NO, playing a crucial role in endothelial XO and XDH expression. Because H_2O_2 can stimulate further production of ROS, the stimulation of conversion from XDH to XO can be

regarded as a feed-forward mechanism. What's more, it was also found that the calcium ionophore A23187 caused XDH-to-XO conversion, which could be blocked by 2-APB and NO donors and induced by thapsigargin and M-3M3FBS [28]. Recent research also found that eNOS deficiency is associated with an up-regulation of XOR facilitating the nitrate-nitrite-NO pathway and decreasing the generation of ROS. Therefore, NO homeostasis which can regulate blood pressure maybe related with the level of XOR, and regulation of XOR would be a potential factor to influence the blood pressure [29].

On the other hand, there are a few genes and proteins which can restrict the human XOR expression, such as E-box and TATA-like elements and a number of transcriptional proteins, including AREB6-like proteins and DNA-dependent protein kinase (DNA-PK). SAFB1 and AREB6-like proteins suppress transcription of XOR by binding to and direct interacting with the E-box, DNA-PK, and tumor suppressors. Because oncostatin M (OSM) increases the phosphorylation of SAFB1, so it can increase hXOR mRNA expression. However, the expression is significantly inhibited by silencing the DNA-PK catalytic subunit or SAFB1 expression [30].

It was reported that gene expression of XOR may be regulated by nutritional factors, oxygen tension, steroid hormones, phorbol esters, regenerative and hyperplastic stimulus, different cytokines implicated in inflammation, insulin, growth factors and so on. Various inflammatory cytokines, including IFNγ, IL-1, IL-6, and TNF, and steroids may up-regulate XOR expression [8]. In recent years, it has been showed that autophagy can also up-regulate the expression of XO and then enhance the early ROS production. The molecular silencing of autophagy-dependent ATG genes (ATG5, ATG7, and LC3) and the pharmacologic inhibition of autophagy with3-MA and wortmannin reduced ROS production significantly. Accordingly, due to the inhibition of cathepsin S to autophagy, ROS generation, DNA damage, and cell death reduced [5, 31].

6. Changes in Tumors

6.1. Changes in Tumors

XOR is down regulated in many tumors, including breast tumors [32] gastric cancer [33], colorectal cancer [34], lung cancer [35] and serous ovarian cancer [36]. In recent years, Nina et al pointed that XOR was down regulated in 64% of the tumors as compared to the corresponding normal tissues [26].

Studies showed that there is no difference between prostate cancer and the other localized and metastatic diseases in oxidative stress indexes, however, metastatic disease showed a short-time lag preceding oxidation and increased malonyldialdehyde indicating a state of high oxidative stress [37]. What's more, patients with metastatic diseases are more likely to catch cancer. It was found that the overall incidence of cancer was significantly higher among gout patients than control group [38].

6.2. Relation with Genesis, Development and Prognosis of Tumors

Many researchers have confirmed that there is an obvious relation between the decreased activity of XO or XOR and tumors. It was shown that there is a qualitative relationship existing between the % XO activity and tumor size for some tumors. Indeed, in the CaNT tumour a maximum is followed by a decrease in % XO activity and the % XO activity of the SaF tumour does not continue to rise while the necrotic volume increases steadily [39].

Decreased XOR was not only associated with large tumor size, but also with the advanced stage, deep tumor penetration, diffusely spread tumor location, positive lymph node status, non-curative disease, cellular aneuploidy, high S-phase fraction and high cyclooxygenase-2 expression [33].

Additionally, the absence of XOR is an independent predictor of bad outcome in many patients with cancers, such as the patients with breast cancer [24] and those with NSCLC received adjuvant chemotherapy [33]. Clinical studies indicated that Patients with high XOR frequency had a longer median survival day than those with low XOR frequency. What's more, patients with loss of XOR have twice the risk of distant recurrence as compared with those with a moderately decreased or normal expression [36]. This may be because low XOR expression was significantly associated with time to tumor relapse [35]. The polymorphisms of XDH of patients may also be used to explain the difference of survival of patients genetically. Clinical studies with 470 women with primary breast cancer indicated unfavorable genetic variants in the rs207454 (XDH) and rs3736729 (GCLC) polymorphisms were significantly associated with disease free survival and overall survival and may act as predictors of the outcome in negative progesterone receptor and negative estrogen receptor breast cancer patients, respectively[40].

Collectively, decreased XOR or XO may be a marker for more aggressive tumor biology involved in genesis, development and prognosis of tumors.

6.3. Regulation on Tumors

Many researchers tried to find the mechanism of the effect of decreased XOR on tumors. It has reported that XO can enhance the production of hydrogen peroxide, but it has poor effect on cell death, because allopurinol did not protect cell against death, additionally, the siRNA against XO also did not present this function [13]. However, controversial results have been obtained in another group. Masaharu et al [41] clarified that H_2O_2, which is one of the reactive oxygen species (ROS) by XO, induces apoptosis and affects extravillous trophoblast (EVT) function due to significantly inhibiting the invasion ability, tube-like formation and HIF1A and ITGAV of TCL1.

Additionally, it has been reported that low levels of both XDH and cellular retinol binding protein play a crucial role

in retinoic acid deficiency in malignant human mammary epithelial cells. Indeed, xanthine dehydrogenase (XDH) is capable of oxidizing both t-ROL bound to the CRBP and all-trans-retin aldehyde (t-RAL) to all-trans-retinoic acid (t-RA) [16, 42], which can induce the epithelia differentiation [43], therefore, low level or activity of XDH combined with lack of expression of cellular retinol binding protein (CRBP) contributes to decrease the ability of breast tumor cells to synthesize t-RA [44].

Genetic regulation may play a crucial role in the proliferation and differentiation of tumors. It was reported that XOR can regulate expression of Id1, COX-2, and MMP-1 [45]. Among these genes, Id1 is a signature gene for tumorigenesis which belongs to the basic helix-loop-helix (bHLH) family of transcription factors and is important to mediate breast tumorigenesis [42]. XOR and Id1 have opposing effects on cell proliferation and overexpression of XOR is not only inhibited Id1-induced proliferation but also stimulated differentiation of Heregulin-b1–treated human breast cancer cells in HC11 cells [46]. It is possible that the poor expression of XOR in both the most aggressive human breast cancer and tumors cells decreases this restraint on Id1 expression. On the other hand, Nina et al found that decreased XOR was associated with a poorly differentiated tumor and an abnormal p53 expression, but not with age at diagnosis, FIGO stage, Ki-67 or tumor size. This relation was confirmed in patients whose tumors were highly differentiated and in patients with a small (<1 cm) residual tumor, and in patients whose tumors present a low Ki-67 protein expression [38].

6.4. Function in Antineoplastic Therapy

Chemotherapy is often used to treat patients with tumors. Xanthine oxidase and xanthine dehydrogenase are two different kinds of forms of XOR which can activate antineoplastic agents. It has been showed that XDH would be a more efficient drug activator for doxorubicin (DOX), streptonigrin (STN), and menadione (MD) than XO for both PH7.4 and PH6.0, moreover, the higher rate of oxygen consumption indicated from drug activation by XDH can be through a two-electron reduction of the quinone drug, which would produce twice the number of oxygen radical than that by XO. Additionally, the decrease in the PH from 7.4 to 6.0 leads to the increase in the rate of oxygen consumption for XDH-activated DOX, STN and MD indicated XDH activity for the reduction of them may be observed at a more acidic PH [47].

Due to high efficiency and less toxicity, the ancient herbal medicines may provide the better strategies for treatment of tumors. Berberine, which is a kind of novel phytochemicals, has been confirmed to induce apoptosis of human prostate cancer cells (PC-3) through ROS medication. The function is initialed from ROS generation, then it occurs the downstream of cytochrome c and Smac/DIABLO from mitochondria and cleavage of caspase-9, caspase-3 and PARP proteins, which have been proved to contribute apoptosis by some agents [48, 49], On the other hand, because it does not induce oxidative stress in the normal prostate epithelial cells, which usually occurs in pathogenesis of many chronic diseases including cancer, thus Berberine would be less harmful [50].

Radiotherapy is another method to cure tumor, however, it usually has the side effects including erythema, individual radiosensitivity and so on. It has been confirmed that there are four genes including in TGFB signaling (SKIL, EP300, APC, and AXIN1) that might have a great impact on the individual radiosensitivity [51-54]. It can be seen from the observation that single nucleotide polymorphism (SNP) affecting DNA repair and TGFB1 signaling plays an important role in the side effect of erythema and individual radiosensitivity, but there is little effect on ROS pathway [55].

7. Relation with the Other Pathology

7.1. Chronic Hypoxia

It was reported that the ratio of the XO activity to the combined XO+XDH activities (% XO activity) is a index for hypoxia in normal tissues and tumors [37]. Sequently, it was showed under chronic hypoxia condition, extracellular superoxide dismutase (EC-SOD) activity is destroyed, which can inhibit the upregulation of redox-sensitive genes including the transcription factor, early growth response-1 (Egr-1), and its downstream gene target, tissue factor (TF),thus growth response-1 (Egr-1) in hypoxia is regarded as the important redox-regulated genes [56]. Furthermore, it was confirmed that hypoxia itself plays an minor role in either Egr-1 expression or O2.- production, indeed, it was extracellular ROS together with activation of ERK1/2 by phosphorylation that regulate the increase in Egr-1 which plays an important role in pulmonary vascular remodeling [57].

Another contribution of XOR to hypoxia was described in chronic intermittent hypoxia (IH), which can cause sleep-disordered breathing with recurrent apnea. The mechanism of positive feed forward ROS-induced ROS can be considered as followed: firstly, IH activated xanthine oxidase (XO) by increased proteolytic conversion of xanthine dehydrogenase to XO, secondly, ROS generated by XO activated calpains, which contributed to HIF-2a degradation by IH. During this process, HIF-2a degradation induced by Calpain involves C-terminus of the HIF-2a protein [31].

7.2. Renal Injury

Using Histological analysis, Toshio et al [58] showed that deposition of crystals and lipid-rich substances contributes to the renal tubular damage in XOR-/- mice. What's more, the level of lipogenesis-related gene expression, adipogenesis-related gene expression, urinary excretions of xanthine and hypoxanthine were significantly elevated. Additionally, various markers of fibrosis, inflammation, ischemia, and oxidative stress were increased in XOR-/-mice, thus the transformation of primary renal epithelial cells from XOR-/- mice to myofibroblasts might induce interstitial fibrosis after epithelial mesenchymal transition (EMT) and ultimately cause renal failure

7.3. Hypertension

It was found that genetic variation in XOR might influence the changes in BP and thus it's an indicator that predicts the incidence of the hypertension. The result shows that pulse pressure increased approximately 2 mmHg more in minor allele carriers of rs11904439, mean arterial pressure and DBP increased about 1 mmHg less in minor allele carriers of rs2043013. what's more, In 2050 hazard ratios in minor allele carriers vs. nonminor allele carriers were 1.31 and 1.69 for rs11904439 and rs148756340, respectively [59]. The relation between genetic variation in XOR and incidence of hypertension has not been elucidated yet, it's possible that XOR could influence the metabolism of the NOs, which is a substance dilate blood vessels and then regulate the BP.

8. Conclusions

This review underlines the physiochemical characteristic, organ distribution, regulation of activity and gene expression, relation with tumors and the other diseases. Apparently, XOR is an important functional component of differentiation and apoptosis whose diminished expression contributed to tumor aggressiveness, and also regarded as both a tumor biomarker for prognosis and a target for tumor treatment. Therefore, we could carry out gene screening XOR in order to find people with the high risk for catching the tumors at the early stage, meanwhile, searching the new strategies to up-regulate XOR should be taken into account. Regulation of XOR gene ad protein is involved in many complicated mechanism, because XOR participates in lots of pathological conditions. Besides various tumors, it is also related with other diseases such as chronic hypoxia, hypertension, and so on. Substances that could up-regulate the expression of XOR also have some side effect on patients with the other diseases, for example, steroid hormones can increase the expression of XOR, but also can aggravate hypertension, therefore, we should figure out the multiple effects of antineoplastic therapy.

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