Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue **S-6 Year 2023** Page **2026:2032***

Targeting Nox4 and Interleukin-6 crosstalk can be a potential strategy for Gastric Cancer prevention

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Article History

Received: 28 September 2023
Revised: 21 October 2023
Accepted: 02 November 2023

Abstract:

Over 950000 million people are being affected in Gastric Cancer (GC) in each year. And so many patients are dying for its lethality. That means prognosis and treatment strategies are not well enough for its treatment. So, in the present scenario research work on this disease is truly significant and necessary. Nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase or NOX) is a family of enzyme consists of seven NOX isoforms members, such as- NOX1, NOX2, NOX3, NOX4, NOX5 and dual oxidase (DUOX) 1 and 2, that possess similarities in terms of enzyme function and structure. All these seven isoforms are transmembrane proteins (having six transmembrane domains) with a NADPH binding site, a FAD-binding site and four heme-binding histidines. Among these enzymes (NOX family), NOX4 is highly expressed in Gastric Cancer (GC) cells. As well as it was observed that high expression of NOX4 is correlated with worse overall survival (OS) in all GC patients. Interestingly, high mRNA expression of NOX4 indicates a worse OS in stage III/IV GC patients, but not in stage I/II GC patients. That is suggesting, NOX4 may contribute to the GC progression and play a crucial role in its (GC) late-stage. And it is observed that NOX4 is related with the cell invasion by regulating the JAK2/STAT3 signaling pathway. Functionally this enzyme family is leading with production of Reactive Oxygen Species (ROS) by utilizing NADPH. Few common examples of

CC License CC-BY-NC-SA 4.0	ROS are hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and hydroxyl radicles (OH) etc. These NOX producing ROS are connected to carcinogenesis as it is involved with so many cellular processes like cell proliferation, DNA damage and angiogenesis etc. On the other hand, recent studies show that Interleukin-6 (IL-6) is significantly related with the cell invasion and JAK2/STAT3 activation. That means both compounds (NOX4 and IL-6) are related with JAK2/STAT3 signaling pathway. And this JAK2/STAT3 signaling actually activates those genes which are associated with cell proliferation and anti-apoptosis. So, it is suggested that, targeting both Nox4 and IL-6 may be a fruitful strategy in GC treatment

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Introduction:

Gastric cancer (GC) is among the most common cancers in the world (You et al., 2021). And GC is highly lethal form of cancer (Yuan et al., 2013). Few very common symptoms like dyspepsia, anorexia, weight loss, and abdominal pain etc. are observed in case GC patients, for this reason GC detection is too hard to detect. In this purpose surgery and chemotherapy are being used as treatments (Keshavjee et al., 2022). ROS or reactive oxygen species are those elements which have extra electron and are produced as metabolic byproducts (Brandes et al., 2014). There are many ways in the cells by which ROS can be produced. When electrons escape from one complex of Electron Transport Chain in mitochondria then ROS are produced. As well as NADPH oxidase (NOX) protein family can produce ROS (Vodjgani et al., 2020). Hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and hydroxyl radicles (OH) are few common examples of ROS (Lambeth & Neish, 2014) ROS have huge significance in immune system through T cell functioning. And it's (ROS) level affects the immune system. A minimal increase of ROS may lead to normal immune function and moderate level of ROS is leading with bacterial defense and chemotaxis but high level of ROS results damage of proteins, lipids, carbohydrates, nucleic acid etc. Moreover, inside the cell few enzymes like superoxide dismutase (SOD), peroxidases, catalases etc. eliminate these oxidizing species so that level of ROS can be restricted in the certain non-toxic level. Apart from ROS, there are many ways of developing tumors including gastric cancer, such as epigenetic factors (Paul et al., 2014). But in case of cancer cells concentration of ROS is very high (Nakamura & Takada, 2014) as well as enzymes from NOX family are also high compare to normal cells (Moloney & Cotter, 2018).

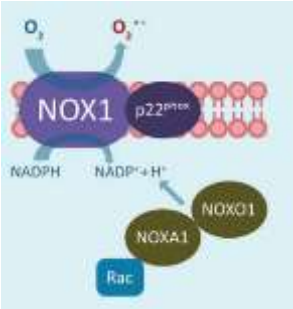
Interleukin-6 (IL-6) is one of the multifunctional cytokine which plays a vital role in host defense. The IL-6 gene is located on the chromosome 7p2 in the human genomes (Simpson et al., 1997). This gene has four introns and five exons. Interleukin-6 is expressed with high concentration in tumor micro-environments for all types of cancer. And it has few functions which may lead to cancer (Brandes et al., 2014).

Basic structure and activation of NOX:

NOX enzymes are major cause for ROS production in the cells. Like as animals, this family of enzyme is also expressed in plants and lower group of organisms. In mammalian NOX family NOX-1, NOX-2, NOX-3, NOX-4, NOX-5, DUOX-1 and 2 enzymes are present. NOX are transmembrane proteins having two state – active and inactive. As a naive monomer it is inactive but with the cytosolic proteins as interaction partners it gets active and stabilized. Enzymes from this family use different strategies for activation (Brandes et al., 2014). Nox1 to Nox4 interact with p22phox in activation purpose, but activation of NOX5 is p22phox independent. p22phox acts as a scaffold protein and functions for the maturation of active NOX protein. However, it gives a proline-rich region which is a binding platform for the cytosolic activator proteins of NOX1-3. In case of DUOX1 and 2, they require DUOXA1 and DUOXA2 as a framework for their maturation and appropriate function.

Number	NOX enzyme	Activating agents
1	NOX1, NOX2, NOX3	Cytoplasmic activator proteins and GTPase RAC
2	NOX4	Constitutive expression
3	NOX5, DUOX 1 and 2	Calcium

Table.1. Activating agents of NOX

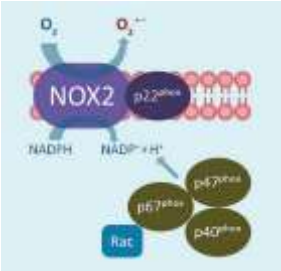


NOX1 Activation:

The expression of NOX1 is significantly elevated in the gastrointestinal tract's epithelial cells. NOXA1 and N1 are needed for NOX1 p22phox activation. In fact, p22phox plays a critical role in the Nox1-dependent O2- (superoxide) production process. Nox1 and p22phox also form a complex. (Vemot et al., 2021).

Fig.1. Activation of NOX1

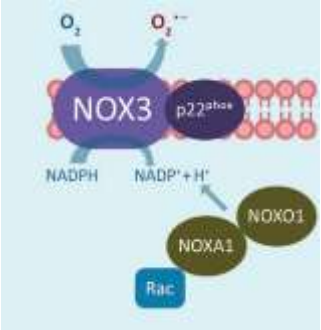
NOX2 Activation:



Like as NOX1, NOX2 also needs p22phox and other cellular proteins like Rac, p67phox, p47phox etc. for its activation. It (Nox2) interacts initially with the p67phox. But this interaction is not well enough for NOX2 activation. Actually, p47phox binds with p22phox that impends p67phox to NOX2 (Vemot et al., 2021).

Fig.2. Activation of NOX2

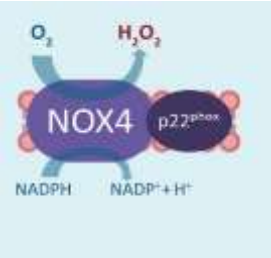
NOX3 Activation:



The expression of NOX3 is restricted to the inner ear and this enzyme is required for the formation of otoliths. And p22phox is also required for the maturation of NOX3 (Vemot et al., 2021).

Fig.3. Activation of NOX3

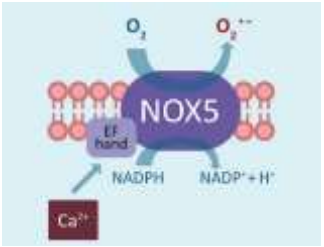
NOX4 Activation:



Nox4 forms a stable complex with the p22phox, though the interaction is different from that of NOX-1 and NOX-2. The NOX4 activity is being thought to be constitutive and by this way mRNA formation regulates the ROS production, though the translation efficiency, mRNA stability and protein stability of Nox-4 are controlled as well (Vemot et al., 2021).

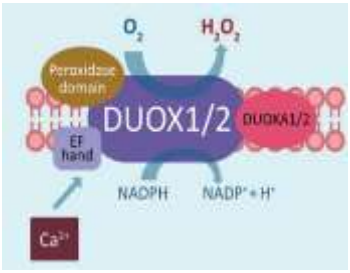
Fig.4. Activation of NOX4

NOX5 Activation:



Unlike NOX-1 to 3, NOX-5 activity is dependent on calcium and number of phosphorylation sites, control ROS formation by this enzyme (Vemot et al., 2021).

Fig.5. Activation of NOX5



DUOX1/2 Activation:

Both DUOX1 and DUOX2 have almost same structure like as NOX5 but these two proteins contain an extracellular peroxidase domain. And DUOXA1/2 are also required as cofactors (Vemot et al., 2021).

Fig.6. Activation of DUOX1/2

Expression of NOX enzyme in different areas of body:

Enzymes	Sites of expression
NOX1	Colon
NOX2	Neutrophils
NOX3	Inner ear
NOX4	Endothelial lining, Stomach
NOX5	Lymphoid tissue
DUOX1 and 2	Thyroid
Interleukin-6	Tumor micro environment

Table.2. Expression of NOX in different areas of body (Gao et al., 2017).

Role of NOX4 in Gastric cancer:

In several cancer cell lines, it was observed that NOX4 is involved in cancer development. In GC cells, MGC-803 and BGC-823 cells NOX4 is associated with regulation of cell proliferation, invasion and migration (Tang et al., 2018). Detail view of these activity is as follows-

Cell proliferation and invasion:

It was found that NOX4 expression was connected with tumour size and prognosis in GC based on clinical data from 90 patients. An in vitro investigation verified that the expression of Cyclin D1, BAX, and other proteins was inhibited when NOX4 was knocked down. Interestingly, NOX4 activated the GLI1 pathway to enhance cell proliferation. Research on GC cell proliferation shows that NOX4 is essential for the growth of gastric cancer cells by producing ROS and then activating the GLI1 signaling pathway (Mengie Ayele et al., 2022).

Metastasis:

The gene expression linked to cell division can be linked to the JAK2/STAT3 signaling pathway. Furthermore, an excess of JAK2/STAT3 signaling may lead to the development of cancer. Large amounts of data from recent research suggest that the overactive JAK2/STAT3 pathway encourages carcinogenesis, tumour growth, cancer cell survival, and solid tumour metastasis. Moreover, the overexpression of the JAK2/STAT3 signaling pathway in GC may be brought on by the overexpression of NOX4 (Vogel et al., 2015).

Angiogenesis:

ROS play an important role in angiogenesis. The ROS-producing NOX family is highly expressed in endothelial cells and is involved in the angiogenesis. It is noted that Nox4 can mediate tumor angiogenesis, by this way NOX-4 is involved in this process (Kumari et al., 2016).

Role of Interleukin-6 in Gastric Cancer:

Interleukin-6 is high in tumor micro environments in all cancers. In case of GC patients, Interleukin-6 (IL-6) is capable to increase the invasive ability and activation of JAK2/STAT3 of the MGC-803 and BGC-823 cells (GC cell lines). And this JAK2/STAT3 signaling is implicated in tumor formation as well as metastatic progression (Gao et al., 2017).

Relation between NOX4 and IL-6 in GC through JAK2/STAT3:

The gene expression linked to cell division can be linked to the JAK2/STAT3 signaling pathway. Furthermore, an excess of JAK2/STAT3 signaling may lead to the development of cancer. Large amounts of data from recent research suggest that the overactive JAK2/STAT3 pathway encourages carcinogenesis, tumour growth, cancer cell survival, and solid tumour metastasis. Moreover, the overexpression of the JAK2/STAT3 signaling pathway in GC may be brought on by the overexpression of NOX4. Then STAT3 undergoes phosphorylation by JAK2, causing the STAT3 dimerization. After this event dimerized STAT3 dissociate from the receptor and then translocate to the nucleus. They bind to certain DNA sequences and stimulate transcription of their target genes. It has been discovered that JAK2/STAT3 signalling can be linked to other pathways such as MAPK/ERK and PI3K/AKT/mTOR signalling to carry out specific cellular actions. As previously stated, NOX4 is associated with JAK2/STAT3 signaling, implying that NOX4 and IL-6 are linked in terms of JAK2/STAT3 signaling (Kumari et al., 2016).

Discussion:

NOX4 expression is higher in GC tissues than in normal tissues. Furthermore, the degree of NOX-produced ROS is connected with GC due to their involvement in several cellular processes such as cell proliferation, DNA damage, and angiogenesis. According to a recent study, NOX4 may stimulate the JAK2/STAT3 pathway to increase GC cell invasion. IL-6, on the other hand, is abundant in the tumour microenvironment. It also promotes cancer by controlling numerous signalling pathways. It should be emphasized that IL-6 is also linked to JAK2/STAT3 signalling. That pathway really activates genes involved in cell growth and anti-apoptosis. Finally, it is proposed that targeting both Nox4 and IL-6, rather than just NOX4 or IL-6, may be a beneficial strategy.

Further study:

There is an idea about relationship between NOX4 expression and GC. But how NOX4 produced ROS is connected with JAK2/STAT3 pathway in GC, this is not such clear. So, this topic should be studied for getting more information related to GC.

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