REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Role of Notch Signaling Pathway in Breast Cancer and Chemo-resistance

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Abstract

Despite the advanced treatment strategies, breast cancer remains the second cancer causing mortality across women worldwide. Notch signaling pathway has been found to be emerged in several cancers as well as in acquired and innate drug resistance. Therefore, understanding the role of Notch molecular mechanism could harbor beneficial impact to overcome chemoresistance and developing novel treatment strategies for cancer. Cisplatin (CIS) is an effective chemotherapeutic agent that is widely used against cancer but resistance is frequently occurs with limited therapeutic efficacy. One approach to overcome such unfavorable resistance is using complementary therapies with CIS. Thymoquinone (TQ) is a main compound in the essential oil of *Nigella sativa*. It has potent oncostatic activity by modulation of multiple regulatory pathways. Pentoxifylline (PTX) is a methylated xanthine derivative with remarkable anti-inflammatory and immunomodulatory actions. Combining TQ and PTX with CIS could be a promising strategy to suppress Notch signaling and overcome CIS resistance.

Keywords: Notch; Cisplatin; thymoquinone; Pentoxifylline; Chemo-resistance

1. Introduction

Breast cancer is the second cancer causing mortality across women. GLOBOCAN 2018 estimated the new breast cancer cases to be 2,088,849 with 626,679 deaths among women **(Ferlay *et al.* 2019)**. Breast cancer incidence rate in Egypt were estimated based upon results from International Agency for Research on Cancer (IARC) and found to be about 47% with 18.8% mortality rate among women in 2018 **(IARC 2018)**. Notch signaling is a highly preserved pathway that has a vital role in cellular differentiation, proliferation, renewal, and apoptosis **(Ho *et al.* 2020)**. Latest studies proved implication of Notch in several types of cancers as well as in acquired and intrinsic drug-resistance **(Xiao *et al.* 2019)**. Cisplatin is a chemotherapeutic agent that was discovered in 1965 by *Barnett Rosenberg* and it is effectively used in treating several cancers. CIS belongs to platinum compounds, which act by binding to guanine bases on both strands of DNA **(Muggia *et al.* 2015)**.

Thymoquinone (TQ) is the major active compound of the essential oil of *Nigella sativa*. Several studies outlined its effectiveness in hindering and treating cancer **(Zidan *et al.* 2018)**. Besides, it has anti-inflammatory, immunomodulatory, and anti-oxidant properties **(Majdalawieh *et al.* 2015)**.

pentoxifylline (PTX) is a methylated xanthine derivative drug that was approved for peripheral vascular disorders **(El-Haggar *et al.* 2018)**. PTX fights cancer through its apoptotic, anti-angiogenic, anti-metastatic, and immunomodulatory properties **(Niderla-Bielińska *et al.* 2018)**

2. Molecular Notch signaling pathway

2.1. Canonical notch ligands

In mammals, the canonical Notch pathway is encoded as four trans-membrane receptors (Notch 1-4) with five different trans-membrane ligands; delta-like ligand (DLL 1, 3, and 4) and Jagged ligand (JAG1 and JAG2) that are expressed by the adjacent cells **(Ho *et al.* 2020)** as presented in **Figure (1).**

When Notch extracellular domain (NECD) interacts with the ligand, disintegrin and metalloproteinase (ADAM) dislodges NECD (S2 cleavage) that continues the interaction with the ligand. Afterward, γ-secretase cleaves the remaining part of Notch receptor inside the cell (S3 cleavage) to release Notch intracellular domain (NICD) **(Clara *et al.* 2019)**. Once NICD is free in the cytosol, it binds with various proteins including CBF1 suppressor of hairless and lag-1 (CSL), mastermind-like proteins (MamL), and P300. Subsequently, this entire complex translocates to the nucleus where P300 acts as histone acetylase and initiates expression of Notch-target genes as myelocytomatosis oncogene cellular homolog (Myc), hairy and enhancer of split-1 (Hes1), Hes related family BHLH transcription factor with YRPW Motif 1 (Hey1), cyclin D3, and P21 **(Kovall *et al.* 2017)**

2.2. Non- canonical notch pathway

The exact mediators of the non-canonical pathway are obscure. Consequently, understandng non-canonical Notch could afford valuable impact in several diseases' therapeutics. However and as shown in **Figure (1)**, Wnt/ β-catenin pathway is a substantial regulator for Notch. The level of uncleaved membrane-bound Notch is inversely corrolated with active β-catenin **(Andersen *et al.* 2012)**.

2.3. Notch pathway and cancer

Multiple documents proved the oncogenic role of Notch signaling in various human cancers, including but not limited to, lung, breast, ovarian, renal, endometria, and some hematological malignancies **(Chatterjee *et al.* 2019)**. Notch pathway promotes tumorigenesis through conserving cancer stem cells (CSCs). These population of CSCs displayed higher activity of Notch signaling in comparison with their corresponding differentiated cells. Interestingly, the $Notch^{+}$ cells showed ability to seed tumors unlike $Notch^{-}$ cells, which failed to initiate tumors in xenograft mouse experiments **(Butti *et al.* 2019)**.

Regulation of ligands' expression as well as the downstream effector genes not only plays a role in normal cellular responses but also in cancer development and progression **(D'Souza *et al.* 2010)**. Each Notch ligand and targeted gene has a crucial function in promoting cancer as shown in **Table (1) (Yuan *et al.* 2015)**.

2.4. Notch-targeted therapies

Designing of anti-Notch therapies is rapidly improving. The majority of Notch-targeted therapies aim to block the release of NICD from the membrane **(Zlobin *et al.* 2019)**. As presented in **Figure (2)**, there are several targets to block Notch signaling involving: ligands' expression, ligands' ubiquitination and trans-endocytosis, expression of Notch receptors' themselves, ligand-receptor binding, heterodimer detachment during Notch activation, ADAM cleavage of Notch, ubiquitination and endocytosis of γ-secretase substrate, γ-secretase cleavage of Notch, association of the coactivator complex with Notch and CSL, heterodimerization of Notch transcriptional complexes, Notch post-translational modifications, and expression of Notch-targeted genes **(Clara *et al.* 2019)**.

2.5. CIS and Notch Signaling

Cisplatin is an alkylating chemotherapeutic agent that acts by different molecular mechanisms as shown in **Figure (3)**. Basically, platinum atom of CIS is covalently binds to N^{7} position of purine residues in DNA strands causing intra- and inter-strand crosslinks **(Costa *et al.* 2019)**. The ability of

Table (1): Role of Notch ligand/ target gene in carcinogenesis

Ligand/ target gene	Function
JAG1	Enhances angiogenesis
JAG2	Promote cell survival and proliferation
DLL1	Governing cell fate decisions and cell-to-cell communication
DLL3	Suppressing cell growth by induction of apoptosis
DLL4	Activating NF-κB signaling, which stimulates VEGF
Hes1	Sequence-specific DNA binding transcriptional factor involved in cellular proliferation and differentiation
Hey1	Development of neoplastic vasculature

NF-κB: nuclear factor kappa B and VEGF: vascular endothelial growth factor

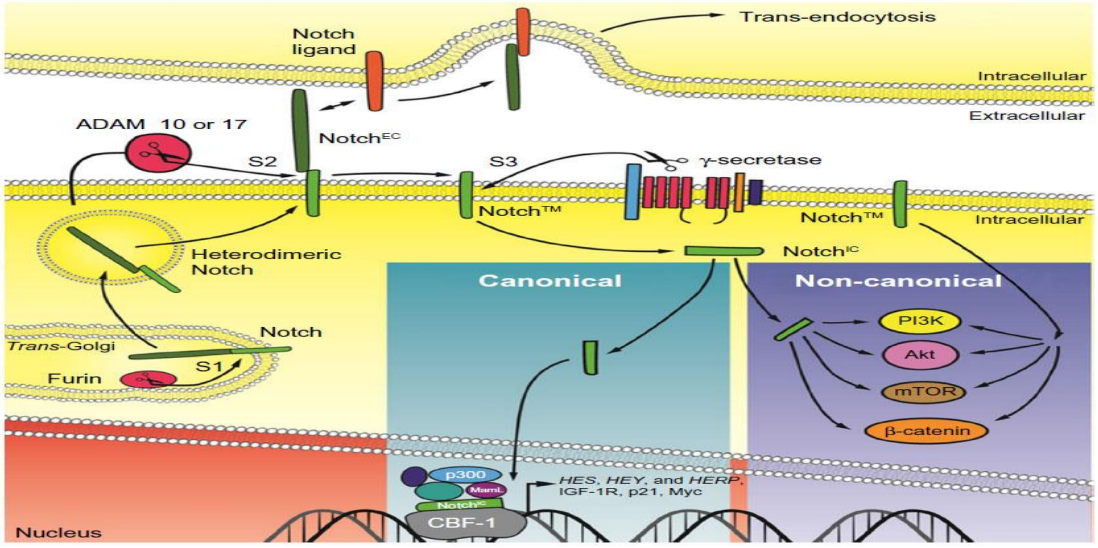

Figure (1): Canonical and non-canonical Notch signaling pathway. In Golgi apparatus, Notch receptor undergoes proteolytic cleavage mediated by furin proteases (S1 cleavage). The receptor is transported to the cell surface membrane. The extracellular domain of Notch (NECD) in the signaling cell binds with the ligands expressed by the neighboring cell. This induces S2 cleavage by ADAM, and releases NECD into the ligand-expressing cell. This is followed by the release of the Notch intracellular domain (NICD) that cleaved by γ- secretase, which cleaves the NICD from the membrane by S3 and S4 cleavages. NICD traverses the nucleus and interacts with the CSL; results in the formation of an active complex with MamL and other co-activators and leads to the transcription of Notch target genes as HES and HEY. Non-canonical ligands activate Notch independently from CSL ligands .ADAM: disintegrin and metalloproteinase, MamL: Mastermind-like protein, CBF1: centromere-binding protein 1, CSL: CBF1 suppressor of hairless and lag-1, HES: hairy and enhancer of split-1, HEY: Hes related family BHLH transcription factor with YRPW motif 1, HERP: homocysteine-induced endoplasmic reticulum protein, IGF-1R: insulin-like growth factor 1 receptor, Myc: myelocytomatosis oncogene cellular homolog, Akt: protein kinase B, PI3K: phosphoinositide 3-kinase, mTOR: mechanistic target of rapamycin **(Barse *et al.* 2015).**

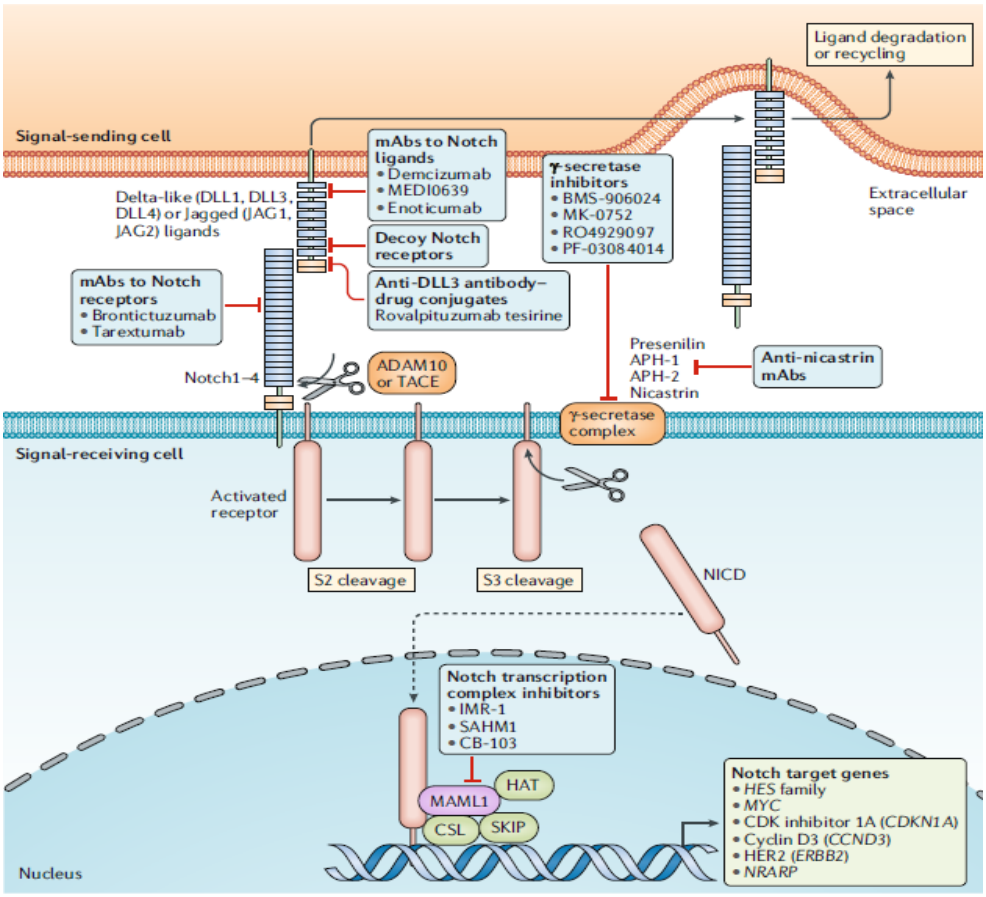

Figure (2): The canonical Notch pathway and related pharmacological inhibitors under investigation. Potential therapeutic agents targeting Notch pathway include monoclonal antibodies (mAbs) targeting Notch receptors or ligands, soluble decoys, ADAM inhibitors, γ- secretase inhibitors, and inhibitors of NICD- interacting transcriptional regulators. NICD: Notch intracellular domain, ADAM10: disintegrin and metalloproteinase domain-containing protein 10, TACE: tumour necrosis factor- α converting enzyme also known as ADAM17, APH: anterior pharynx- defective, HAT: histone acetyltransferase, MAML1: Mastermind- like 1, CSL: CBF1 suppressor of hairless and lag-1, SKIP: ski- interacting protein, HES: hairy and enhancer of split, MYC: myelocytomatosis oncogene cellular homolog, CDK: cyclin dependent kinase, ERBB2: receptor tyrosine-protein kinase, NRARP: Notch-regulated ankyrin repeat protein **(Clara *et al.* 2019)**.

CIS to produce reactive oxygen species (ROS) is another cancer cell killing mechanism. The generated ROS stimulates extracellular signal-regulated kinase (ERK), ataxia telangiectasia mutated (ATM), and P53 pathways. As a result, apoptosis is initiated and cancerous cell death is triggered **(Cao *et al.* 2020)**. Moreover, CIS initiates apoptosis by inhibition of the anti-apoptotic B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xL), whereas it stimulates the pro-apoptotic Bcl-2-associated X protein (Bax) and caspase-9, which facilitates the release of cytochrome c **(Qin *et al.* 2020)**.

Several *in vitro* and *in vivo* studies documented the bility of CIS to down-regulate Notch, JAG1, Hes1 **(Tian *et al.* 2017)**, CTNNB1 with subsequent tumor-eradicating effect **(Yin *et al.* 2018)**.

Unfortunately, by prolonged use of CIS, resistance of tumor cells toward CIS is frequently occurs. There are different mechanisms responsible for CIS resistance; one of them is reverse over-expression of Notch1 **(Tian *et al.* 2017)**. There are several mechanisms to overcome resistance; giving CIS in a drug delivery system formulation, inhibition of metallothionein, using compination therapy with CIS **(Achkar *et al.* 2018)**.

2.6. Thymoquinone as a complementary therapy and Notch modulator

Thymoquinone (TQ) is the fundamental active compound in the essential oil of *Nigella sativa*. Several studies documented its effectiveness in cancer therapy **(Zidan *et al.* 2018)**. The antitumor

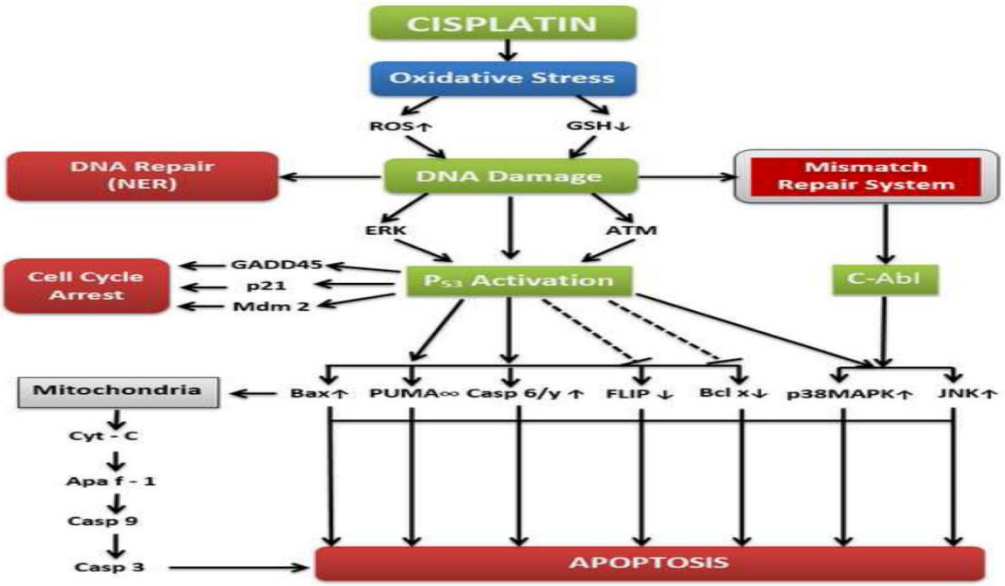

Figure (3): Molecular mechanisms of cisplatin in cancer treatment. ROS: reactive oxygen species, GSH: glutathione, NER: nucleotide excision repair, ERK: extracellular signal-regulated kinase, ATM: ataxia telangiectasia mutated, GADD45: growth arrest and DNA damage 45, Mdm2: mouse double minute 2 homolog, C-Abl: Abelson murine leukemia viral oncogene, Bax: Bcl-2-associated X protein, PUMA: p53 upregulated modulator of apoptosis, Casp: caspase, FLIP: FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein, Bcl xL: B-cell lymphoma extra large, MAPK: mitogen-activated protein kinase, JNK: c-Jun N-terminal kinase, Cyt-C: cytochrome c, Apa f-1: Apoptotic protease activating factor 1 **(Dasari *et al.* 2014)**.

activity of TQ basiclly depends on three main regulatory pathways: apoptosis, cell cycle, and nuclear factor kappa B (NF-κB). Anti-inflammatory action of TQ is linked to inhibition of pro-inflammatory cytokine as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). TQ excets its free radical scavengering activity by up-regulation of the key anti-oxidant enzymes as glutathione reductase (GR) and glutathione-S-transferase (GST) **(Mahmoud *et al.* 2019)**. Previous literature outlined the beneficial using of TQ as an adjuvant with the chemotherapeutics and radiation in order to boosts the therapeutic efficacy as well as limiting the undesired toxicity **(Mahmoud *et al.* 2019)**. Surprisingly, earlier studies reported the inhibitory action of TQ on Notch1, Hes1, and JAG1 and suggested such inhibition is mediated through up-regulation of phosphatase and tensin homologue (PTEN) **(Ke *et al.* 2015, Mu *et al.* 2015)**. In addition, TQ induces CTNNB1 supression by up-regulation of dickkopf Wnt signaling pathway inhibitor 1(DKK1) and cyclin dependent kinase inhibitor 1A (CDNK-1A) **(Kensara *et al.* 2016)**.

2.7. Pentoxifylline as a complementary therapy and Notch modulator:

Pentoxifylline (PTX) is a methylated xanthine derivative drug that was approved for treating peripheral vascular disorders **(Namdar *et al.* 2020)**. Recent studies revealed the anticancer effect of PTX, which could mediated through apoptosis induction by down-regulation of integrins-adhesion molecules **(Niderla-Bielińska *et al.* 2018)** and anti-apoptotic proteins together with up-regulation of death receptors DR4 and DR5. Moreover, PTX impairs DNA-repair mechanis and induces cell cycle arrest at G1-S phase **(Talar *et al.* 2016)**.

From that point, PTX was used as an adjunct drug to enhances the chemotherapeutic action and ameliorates the undesired side effects. For instance, PTX was used with doxorubicin **(Elshazly *et al.* 2016)** and paclitaxel **(Kim *et al.* 2016)** to minimize cardiotoxicity and nephrotoxicity, respictively. Moreover, PTX has been found to supress Notch1 with subsequent Hes1 inhibition **(Niderla-Bielińska *et al.* 2018)**. The anti-inflammatory property could allow PTX to down-regulate JAG1 ligand through inhibition of pro-inflammatory cytokines TNF-α and IL-6 **(El-Haggar *et al.* 2018)**.

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