Communication

The Pathway Flow Starting From Chronic Reactive Oxygen Species Leading to Human Treatment-Resistant Gastric Cancer

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Communication (AOP Report)

The Pathway Flow Starting from Chronic Reactive Oxygen Species Leading to Human Treatment-Resistant Gastric Cancer

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Simple Summary: The research aims to elucidate a pathway starting with increases in cellular reactive oxygen species (ROS) and chronic ROS leading to human treatment-resistant gastric cancer (GC) through Wnt/beta-catenin signaling and epithelial-mesenchymal transition (EMT). The main topic in this article is an Adverse Outcome Pathway (AOP) 298 entitled "Increases in cellular reactive oxygen species (ROS) and chronic ROS leading to human treatment-resistant gastric cancer (GC)" consisting of 2 molecular initiating events (MIEs), "Increases in cellular ROS" and "Chronic ROS", 3 key events (KEs), "porcupine-induced Wnt secretion and Wnt signaling activation", "beta-catenin activation", "epithelial-mesenchymal transition (EMT)" and 1 adverse outcome (AO), "human treatment-resistant GC".

Abstract: The injury causes resistance in human gastric cancer (GC). This Adverse Outcome Pathway (AOP) entitled "Increases in cellular reactive oxygen species (ROS) and chronic ROS leading to human treatment-resistant gastric cancer (GC)" consists of molecular initiating event (MIE) as "Increases in cellular ROS" and "Chronic ROS", followed by series of key events (KEs); "porcupine-induced Wnt secretion and Wnt signaling activation", "beta-catenin activation", "epithelial-mesenchymal transition (EMT), and adverse outcome (AO) as "human treatment-resistant GC" in the sequence. ROS has multiple roles in disease such as development and progression of cancer, or apoptotic induction causing anti-tumor effects. In this AOP, we focus on the role of sustained levels of chronic ROS to induce the therapy-resistance in human GC. EMT, which is cellular phenotypic change from epithelial to mesenchymal-like features, demonstrates cancer stem cell (CSC)-like characteristics in human GC. EMT is induced by Wnt/beta-catenin signaling, providing the rationale to have Wnt secretion and beta-catenin activation as KEs in the AOP.

Keywords: Adverse Outcome Pathway (AOP); epithelial-mesenchymal transition; gastric cancer; reactive oxygen species (ROS); Wnt signaling

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1. Introduction

Molecular signaling pathway networks are regulated in epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs), which exhibit anti-cancer drug-resistant features. To reveal the mechanism of human treatment-resistant gastric cancer (GC) and the relationship between oxidative stress response and GC, analysis on oxidative stress response and molecular networks in diffuse- and intestinal-type GC has been performed. The network pathways of GC were analyzed by Ingenuity Pathway Analysis (IPA). NRF2-mediated oxidative stress response network included molecules related to Regulation of EMT by growth factors pathway and Production of nitric oxide and reactive oxygen species in macrophages such as PI3K and AKT.

This research aims to ensure the safety of therapeutics such as anti-cancer drugs by revealing the molecular mechanisms that contribute to the efficacy and side effects or unexpected and off-targeted adverse effects. Chemicals induce molecular alterations and body responses. Recent progress in cellular and molecular network pathway analysis has revealed the activation mechanism of cellular signal transduction upon cancer and chemical stimulation. In developing anti-cancer drugs such as molecular-targeting therapeutics, identifying target molecules and inhibiting or activating the signaling transduction related to the target molecules is important. Anti-cancer therapeutics targeting Wnt/beta-catenin signaling pathway regulating cell self-renewal, Hedgehog signaling pathway, Notch signaling pathway, and EGFR receptor signaling pathway have been developed and approved, however, off-target effects for molecular network pathways are not fully understood. To elucidate the safety of the molecular-targeted therapeutics and the cellular therapeutics using multipotent stem cells, it is critical to predict the off-target network pathways unexpected. Molecular network pathway analysis utilizing the existing abundant data in databases is needed [1,2]. This study aims to predict the side-effects or adverse effects of the therapeutics by analyzing the molecular network pathway dynamism utilizing the data on databases.

ROS consist of free oxygen radicals such as superoxide, hydroxyl radical, nitric oxide, organic radicals, peroxyl radicals, alkoxyl radicals, thiyl radicals, sulfonyl radicals, thiyl peroxyl radicals, or disulfides, and non-radical ROS such as hydrogen peroxide, singlet oxygen, ozone/trioxygen, organic hydroperoxides, hypochlorite, peroxynitrite, nitrosoperoxycarbonate anion, nitrocarbonate anion, dinitrogen dioxide, nitronium, and highly reactive lipid- or carbohydrate-derived carbonyl compounds [3]. ROS have double-edged effects, which may affect tumorigenesis. ROS play crucial roles in defense of human from infection, whereas excess prolonged ROS cause several diseases including cancer, cardiovascular disease, neurological disease, sensory impairment and psychiatric disease [4]. Nicotinamide adenine diphosphate (NADPH) oxidase catalyzes the production of superoxide by the one-electron reduction of oxygen and produces ROS [5] (Figure 1).

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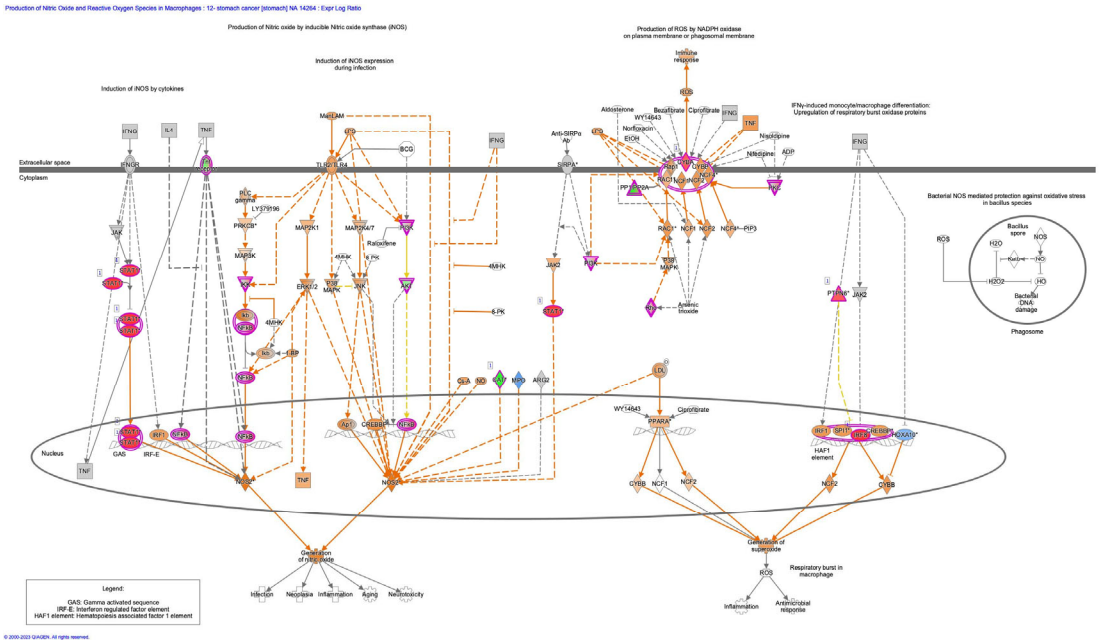

Figure 1. Canonical pathway of production of nitric oxide and reactive oxygen species in macrophages, and its molecular relationship to epithelial-mesenchymal transition (analyzed in Ingenuity Pathway Analysis (IPA)). Analysis of 12- stomach cancer 14264, as of June 2023.

2. Outline of AOP298

2.1. Structure of AOP298

The AOP 298 entitled "Increases in cellular reactive oxygen species (ROS) and chronic ROS leading to human treatment-resistant gastric cancer (GC)" consists of two molecular initiating events; (MIE1; KE1940, Increases in cellular ROS, (MIE2; KE1753, chronic ROS), key events (KEs) as porcupine-induced Wnt secretion and Wnt signaling activation (KE1; KE1754), beta-catenin activation (KE2; KE1755), and epithelial-mesenchymal transition (EMT) (KE3; KE1457), and adverse outcome (AO; KE1651) as human treatment-resistant GC (Figure 2).

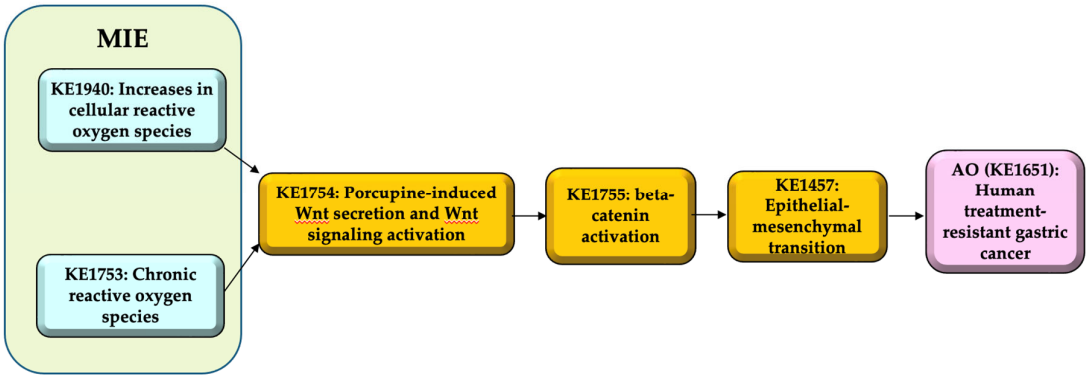

Figure 2. AOP 298 "Increases in cellular ROS and chronic ROS leading to human treatment-resistant gastric cancer".

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While ROS have both benefits and risks in human health, chronic ROS, where excess of ROS prolongs for a long time induces sustained tissue damage, macrophage activation. Porcupine-induced Wnt secretion in macrophages induces proliferation and beta-catenin activation leading to epithelial-mesenchymal transition (EMT). EMT induces cancer migration and drug resistance, causing human treatment-resistant gastric cancer.

AOP298-related information is summarized in Table1.

Table 1. AOP298-related information.

Item	Title
AOP	Increases in cellular reactive oxygen species (ROS) and chronic ROS leading to human treatment-resistant gastric cancer (GC)
MIE1	KE1940: Increases in cellular reactive oxygen species (ROS)
MIE2	KE1753: Chronic reactive oxygen species (ROS)
KE1	KE1754: Porcupine-induced Wnt secretion and Wnt signaling activation
KE2	KE1755: beta-catenin activation
KE3	KE1457: Epithelial-mesenchymal transition
AO	KE1651: Treatment-resistant gastric cancer

2.2. Summary of scientific evidence assessment

2.2.1. MIE1; KE1940: Increases in cellular reactive oxygen species (ROS)

Increases in cellular ROS are observed when cells are exposed to various stressors as allergens, ionizing radiation and chemicals [6]. The ROS includes free radicals (e.g., Superoxide anion, hydroxyl radical, nitric oxide, nitrogen dioxide, organic radicals, peroxyl radicals, alkoxyl radicals, thiyl radicals, sulfonyl radicals, thiyl peroxyl radicals, and disulfide) and non-radical ROS (hydrogen peroxide, singlet oxygen, ozone/trioxygen, organic hydroperoxides, hypochloride, peroxynitrite, nitrosoperoxycarbonate anion, nitrocarbonate anion, dinitrogen dioxide, nitronium, and highly reactive lipid- or carbohydrate-derived carbonyl compounds). Increases in ROS are involved in various diseases.

2.2.2. MIE2; KE1753: Chronic reactive oxygen species (ROS)

ROS play important role in physiological status and diseases. The balance between the production of ROS and detoxification maintains the physiological condition, which is in prolonged excess ROS production in cancer progress [3]. The essentiality of chronic ROS is high in cancer.

2.2.3. KE1; KE1754: Porcupine-induced Wnt secretion and Wnt signaling activation

Sustained tissue damage induces inflammation. Wnt/beta-catenin signaling is essential for intestinal homeostasis, where macrophage-derived Wnt in intestinal repair is crucial for rescuing intestinal stem cells from radiation lethality [4].

2.2.4. KE2; KE1755: beta-catenin activation

The oncoprotein beta-catenin stabilizes and translocates to nucleus, followed by induction of ZEB1 transcription factor that induces epithelial-mesenchymal transition (EMT) [7]. One of the important signaling pathways inducing EMT is the canonical Wnt/beta-catenin pathway, where beta-catenin acts as coactivator of T-cell and lymphoid enhancer (TCF-LEF) factors [8]. Beta-catenin/TCF4 binds to the ZEB1 promoter and induces the transcription leading to EMT, a main hallmark of malignant cells [7].

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2.2.5. KE3; KE1457: Epithelial-mesenchymal transition (EMT)

It is known that EMT plays an important role in therapeutic resistance and drug responses in human gastric cancer [2,9]. EMT is a critical regulator of the CSC phenotype and drug resistance [9]. EMT program is involved in metastasis of gastric cancer [10,11]. Triggering receptor expressed on myeloid cells 2 (TREM2), a key gene in gastric cancer progression, promotes EMT [12].

2.2.6. AO; KE1651: Treatment-resistant gastric cancer

Gastric cancer can be classified into diffuse- and intestinal-type gastric cancer with mRNA ratio of CDH2 to CDH1 [13]. The diffuse-type gastric cancer, which has a poor prognosis and is treatment-resistant, has up-regulated genes that are involved in EMT compared to intestinal-type gastric cancer [14,15]. Gastric cancer-derived mesenchymal stromal cells-primed macrophages promote metastasis and EMT in gastric cancer [16].

Scientific evidence assessment of AOP298 is summarized as support for the biological plausibility of KERs (Table 2), support for the essentiality of KEs (Table 3), and empirical support for KERs (Table 4).

Table 2. Support for Biological Plausibility of KERs in AOP298.

Item	Evidence
MIE1 => KE1: Increases in cellular ROS leads to Porcupine-induced Wnt secretion and Wnt signaling activation	Biological Plausibility of the MIE1 => KE1 is moderate. Rationale: Increases in cellular ROS caused by/causes DNA damage, which will alter several signaling pathways including Wnt signaling. ROS stimulate inflammatory factor production and Wnt/beta-catenin signaling [17].
MIE2 => KE1: Chronic ROS leads to Porcupine-induced Wnt secretion and Wnt signaling activation	Biological Plausibility of the MIE2 => KE1 is moderate. Rationale: Sustained ROS increase caused by/causes DNA damage altering several signaling pathways including Wnt signaling. Macrophages accumulate into the injured tissue to recover the tissue damage, which may be followed by porcupine-induced Wnt secretion. ROS stimulate inflammatory factor production and Wnt/ beta-catenin signaling [17].
KE1 => KE2: Porcupine-induced Wnt secretion and Wnt signaling activation leads to beta-catenin activation	Biological Plausibility of the KE1 => KE2 is moderate. Rationale: Secreted Wnt ligand stimulates Wnt/beta-catenin signaling, in which beta-catenin is activated. Wnt ligand binds to Frizzled receptor, which leads to GSK3beta inactivation. GSK3beta inactivation leads to beta-catenin dephosphorylation, which avoids the ubiquitination of the beta-catenin and stabilize the beta-catenin [8]
KE2 => KE3: beta-catenin activation leads to Epithelial-mesenchymal transition (EMT)	Biological Plausibility of the KE2 => KE3 is moderate. Rationale: Beta-catenin activation, which includes stabilizing the dephosphorylated beta-catenin and translocation of beta-catenin into the nucleus, induces the formation of beta-catenin-TCF complex and transcription of transcription factors, such as Snail, Zeb and Twist [11,18–21]. EMT-related transcription factors including Snail, ZEB, and Twist, are up-regulated in cancer cells [22]. The transcription factors such as Snail, ZEB, and Twist bind to E-cadherin (CDH1) promoter and inhibit the CDH1 transcription via the consensus E-boxes (5′-CACCTG-3′ or 5′-CAGGTG-3′), which leads to EMT [22].
KE3 => AO: Epithelial-mesenchymal transition (EMT) leads to	Biological Plausibility of the KE3 => AO is moderate. Rationale: Some population of the cells exhibiting EMT

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human treatment-resistant gastric cancer	demonstrates the feature of cancer stem cells (CSCs), which are related to cancer malignancy [9,23–25]. EMT phenomenon is related to cancer metastasis and cancer therapy resistance [26,27]. The increase in expression of enzymes that degrade the extracellular matrix components and the decrease in adhesion to the basement membrane in EMT induce the cell to escape from the basement membrane and metastasis [27]. Morphological changes observed during EMT are associated with therapy resistance [27].

Table 3. Support for Essentiality of KEs in AOP298.

Item	Evidence
KE1: Porcupine-induced Wnt secretion and Wnt signaling activation	Essentiality of the KE1 is moderate. Rationale for Essentiality of KEs in the AOP: The Wnt signaling activation is essential for the subsequent beta-catenin activation and cancer resistance.
KE2: beta-catenin activation	Essentiality of the KE2 is moderate. Rationale for Essentiality of KEs in the AOP: beta-catenin activation is essential for the Wnt-induced cancer resistance.
KE3: Epithelial-mesenchymal transition (EMT)	Essentiality of the KE3 is moderate. Rationale for Essentiality of KEs in the AOP: EMT is essential for the Wnt-induced cancer promotion and acquisition of resistance to anti-cancer drug.

Table 4. Empirical support for KERs in AOP298.

Item	Evidence
MIE1 => KE1: Increases in cellular ROS leads to Porcupine-induced Wnt secretion and Wnt signaling activation	Empirical Support of the MIE1 => KE1 is moderate. Rationale: Production of ROS and DNA double-strand break causes the tissue damages [28]. ROS-related signaling induces Wnt/beta-catenin pathway activation [29].
MIE2 => KE1: Chronic ROS leads to Porcupine-induced Wnt secretion and Wnt signaling activation	Empirical Support of the MIE2 => KE1 is moderate. Rationale: Production of ROS and DNA double-strand break causes the tissue damages [28]. ROS-related signaling induces Wnt/beta-catenin pathway activation [29].
KE1 => KE2: Porcupine-induced Wnt secretion and Wnt signaling activation leads to beta-catenin activation	Empirical Support of the KE1 => KE2 is moderate. Rationale: Dishevelled (DVL), a positive regulator of Wnt signaling, form the complex with FZD and lead to trigger the Wnt signaling together with Wnt coreceptor low-density lipoprotein (LDL) receptor-related protein 6 (LRP6) [19,30]. Wnt binds to FZD and activate the Wnt signaling [19,31,32]. Wnt binding towards FZD induce the formation of the protein complex with LRP5/6 and DVL, leading to the down-stream signaling activation including beta-catenin [8].
KE2 => KE3: beta-catenin activation leads to Epithelial-mesenchymal transition (EMT)	Empirical Support of the KE2 => KE3 is moderate. Rationale: The inhibition of c-MET, which is overexpressed in diffuse-type gastric cancer, induced increase in phosphorylated beta-catenin, decrease in beta-catenin and Snail [11].

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KE3 => AO: Epithelial-mesenchymal transition (EMT) leads to human treatment-resistant gastric cancer	The garcinol, which has an anti-cancer effect, increases phosphorylated beta-catenin, decreases beta-catenin and ZEB1/ZEB2, and inhibits EMT [18]. The inhibition of sortilin by AF38469 (a sortilin inhibitor) or small interference RNA (siRNA) results in a decrease in beta-catenin and Twist expression in human glioblastoma cells [21]. Histone deacetylase inhibitors affect EMT-related transcription factors including, ZEB, Twist, and Snail [33]. Snail and Zeb induces EMT and suppress E-cadherin (CDH1) [22,34,35]. Empirical Support of the KE3 => AO is moderate. Rationale: EMT activation induces the expression of multiple members of the ATP-binding cassette (ABC) transporter family, which results in the resistance to doxorubicin [9,36]. TGFbeta-1 induced EMT results in the acquisition of cancer stem cell (CSC) like properties [9,37]. Snail-induced EMT induces cancer metastasis and resistance to dendritic cell-mediated immunotherapy [38]. Zinc finger E-box-binding homeobox (ZEB1)-induced EMT results in the relief of miR-200-mediated repression of programmed cell death 1 ligand (PD-L1) expression, a major inhibitory ligand for the programmed cell death protein (PD-1) immune-checkpoint protein on $CD8^+$ cytotoxic T lymphocyte (CTL), subsequently the $CD8^+$ T cell immunosuppression and metastasis [39].

3. Discussion

This AOP298 "Increases in cellular ROS and chronic ROS leading to human treatment-resistant gastric cancer" consists of several components: "Increases in cellular ROS" and "Chronic ROS" as MIEs, "porcupine-induced Wnt secretion and Wnt signaling activation", "beta-catenin activation" and "Epithelial-mesenchymal transition (EMT)" as intermediate KEs, and "Human treatment-resistant gastric cancer" as an AO. The description of the AOP is based on a mechanism of drug resistance, metastasis, and gastric cancer progression, of which application would be the risk assessment of anti-cancer drugs and the development of the anti-cancer treatment as well.

The chronic low-level increased ROS play crucial role in the development of radioresistant GC via tumor microenvironment alteration and EMT [40]. Radiation causes promotion of metastasis of cancer via ROS and EMT [41]. The extent of ROS level seems to be critical in balancing the cancer cell development and cell death [42].

4. Conclusions

The AOP298 "Increases in cellular ROS and chronic ROS leading to human treatment-resistant gastric cancer" illustrates a pathway beginning at chronic increases of ROS inducing Wnt signaling activation and leading to EMT and treatment-resistant GC in human. The description includes a mechanism of drug resistance, metastasis, and GC progression, of which application would be the risk assessment of anti-cancer drugs and the development of the anti-cancer treatment as well.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Document S1: PDF snapshot of AOP298 (298-2024-06-02T23-53-20+00-00).

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

The information on AOP298 can be downloaded at: https://aopwiki.org/aops/298. This AOP report article is for summarizing the content of the AOP298 and scientific review as a part of the OECD AOP project: https://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm.

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