Reviewer 1

Major (Round 1)

Reviewer comments

This AOP Report addresses a highly important area of cancer biology, seeking to connect the environmental stress of chronic reactive oxygen species (ROS) accumulation with the critical clinical outcome of treatment resistance in gastric cancer. The manuscript is well-written and the overall argument for a mechanistic link is compelling and supported by existing literature (as evidenced by the reference list). The work is suitable for publication in Cancers pending the clarification and strengthening of the specific pathway details outlined in the major comments below.

Major comments:

1. As an AOP (Adverse Outcome Pathway) report, the pathway must be defined with clear, distinct steps. The current description is broad ("The pathway flow starting..."). The authors should explicitly define the Key Events (KEs) and the Key Event Relationships (KERs) that constitute this pathway. For the MIE, is this "increase in ROS" or a specific molecular target (e.g., oxidation of a specific protein/lipid)? What are the key cellular changes (e.g., specific transcription factor activation, EMT, cancer stem cell induction) that bridge chronic ROS to resistance? Clearly define the "treatment resistance." Is it resistance to 5-FU, platinum-based therapy, radiation (as suggested by Ref 41), or a general multi-drug resistance phenotype?
2. The concept of "chronic ROS" is central to the hypothesis, distinguishing it from acute oxidative stress. This distinction needs a robust definition and justification. How is the level of ROS characterized as 'chronic' vs. 'acute'? Please discuss the specific cellular adaptations (e.g., upregulation of antioxidant systems like Nrf2 or changes in mitochondrial function) that maintain this chronic state.
3. The manuscript should expand on how chronic ROS in the cancer cells *transforms* the surrounding TME (e.g., induction of immunosuppression, promotion of angiogenesis, activation of fibroblasts). Clarifying this intercellular signaling mechanism would significantly strengthen the pathway model.
4. Are there specific ROS-sensitive targets or signaling nodes unique to gastric cancer that are not seen in, say, colorectal or pancreatic cancer? If so, highlight these unique features to justify the focus. If not, the authors should temper the title/abstract to reflect a pathway applicable to gastrointestinal cancers or solid tumors in general, before detailing its application to gastric cancer.

Authors' point-to-point responses

Comments 1: This AOP Report addresses a highly important area of cancer biology, seeking to connect the environmental stress of chronic reactive oxygen species (ROS) accumulation with the critical clinical outcome of treatment resistance in gastric cancer. The manuscript is well-written and the overall argument for a mechanistic link is compelling and supported by existing literature (as evidenced by the reference list). The work is suitable for publication in Cancers pending the clarification and strengthening of the specific pathway details outlined in the major comments below.

1. As an AOP (Adverse Outcome Pathway) report, the pathway must be defined with clear, distinct steps. The current description is broad ("The pathway flow starting..."). The authors should explicitly define the Key Events (KEs) and the Key Event Relationships (KERs) that constitute this pathway. For the MIE, is this "increase in ROS" or a specific molecular target (e.g., oxidation of a specific protein/lipid)? What are the key cellular changes (e.g., specific transcription factor activation, EMT, cancer stem cell induction) that bridge chronic ROS to resistance? Clearly define the "treatment resistance." Is it resistance to 5-FU, platinum-based therapy, radiation (as suggested by Ref 41), or a general multi-drug resistance phenotype?

Response 1: Thank you for pointing this out. We agree with this comment. Therefore, we have changed the title to "Adverse Outcome Pathway (AOP) 298 on increase in reactive oxygen species (ROS) leading to human treatment-resistant gastric cancer" from "The pathway flow starting from chronic reactive oxygen species leading to human treatment-resistant gastric cancer" in the revised manuscript. The KEs and the KERs have

been defined with the latest information in the Abstract and Section 2. Outline of AOP298 in the revised manuscript. “Treatment resistance” has been defined as resistance to drugs, therapy, radiation, etc. (line 30 in the revised manuscript.)

Comments 2: The concept of "chronic ROS" is central to the hypothesis, distinguishing it from acute oxidative stress. This distinction needs a robust definition and justification. How is the level of ROS characterized as 'chronic' vs. 'acute'? Please discuss the specific cellular adaptations (e.g., upregulation of antioxidant systems like Nrf2 or changes in mitochondrial function) that maintain this chronic state.

Response 2: We agree, and have accordingly revised Section 3. Discussion to emphasize this point. In particular, the sentence “Specific cellular adaptations—e.g., up-regulation of antioxidant systems such as Nrf2 or changes in mitochondrial function—may maintain the chronic state” was added. (lines 205-207 in the revised manuscript.)

Comments 3: The manuscript should expand on how chronic ROS in the cancer cells transforms the surrounding TME (e.g., induction of immunosuppression, promotion of angiogenesis, activation of fibroblasts). Clarifying this intercellular signaling mechanism would significantly strengthen the pathway model.

Response 3: We agree, and have accordingly revised the discussion to emphasize this point with additional references: “The tumor microenvironment (TME), consisting of immune cells, natural killer cells, the extracellular matrix, etc., plays a critical role in tumor initiation, development, and metastasis by manipulating redox signaling [53,54]. Interactions among tumor-associated macrophages, gastric cancer cells, and natural killer cells induce immune checkpoint molecules that interact with immune cells in the TME of gastric cancer, thereby evading anti-tumor immunity. [55]. Cancer cells use chronic ROS to neutralize, exhaust, and suppress anti-tumor immune cells [53]. Persistent oxidative stress signals from cancer cells transform fibroblasts into pro-tumorigenic cancer-associated fibroblasts [53]. Chronic ROS is crucial for activating the TME in treatment-resistant cancer.” (lines 213-221 in the revised manuscript.)

References:
53. Ranbhise, J.S.; Singh, M.K.; Ju, S.; Han, S.; Yun, H.R.; Kim, S.S.; Kang, I. The redox paradox: Cancer's double-edged sword for malignancy and therapy. Antioxidants (Basel) 2025, 14, doi:10.3390/antiox14101187.
54. Arneth, B. Tumor Microenvironment. Medicina (Kaunas) 2019, 56, doi:10.3390/medicina56010015.
55. Cozac-Szőke, A.R.; Cozac, D.A.; Negovan, A.; Tinca, A.C.; Vilaia, A.; Cocuz, I.G.; Sabău, A.H.; Niculescu, R.; Chiorean, D.M.; Tomuț, A.N., et al. Immune cell interactions and immune checkpoints in the tumor microenvironment of gastric cancer. Int J Mol Sci 2025, 26, doi:10.3390/ijms26031156.

Comments 4: Are there specific ROS-sensitive targets or signaling nodes unique to gastric cancer that are not seen in, say, colorectal or pancreatic cancer? If so, highlight these unique features to justify the focus. If not, the authors should temper the title/abstract to reflect a pathway applicable to gastrointestinal cancers or solid tumors in general, before detailing its application to gastric cancer.

Response 4: Thank you for your insightful comment. Epidemic studies indicate that atomic bomb survivors have a higher rate of gastric cancer. Therefore, we focused on gastric cancer in AOP298 because ROS caused by radiation might be involved in gastric cancer. The following sentence has been added to emphasize the possibility of application of this pathway in other cancers: “The pathway network of gastric cancer and other cancers, with cross-talk among various signaling pathways, would be interesting to investigate in the future.” (lines 228-230 of the revised manuscript.)

Accept (Round 2)

Comments

Well addressed.

Reviewer 2

Major (Round 1)

Reviewer comments

This manuscript presents a well-structured and conceptually valuable Adverse Outcome Pathway (AOP) titled "Increase in Reactive Oxygen Species (ROS) and Chronic ROS Leading to Human Treatment-Resistant Gastric Cancer (GC)". The AOP delineates a mechanistic sequence from molecular initiating events (MIEs)—namely acute and sustained increases in ROS—through a series of biologically plausible key events (KEs), culminating in the adverse outcome of therapy-resistant gastric cancer. But I have several following concerns:

1. In the Introduction section, the statement "The NRF2-mediated oxidative stress response network included molecules related to the Regulation of EMT by growth factors pathway and Production of nitric oxide and reactive oxygen species in macrophages, such as PI3K and AKT" should be supported by relevant literature describing the processes involved in the NRF2-mediated oxidative stress response.
2. Should the Introduction section provide references discussing the regulatory relationship between NRF2 and EMT in gastric cancer?
3. In Table 2, the "Biological Plausibility" is rated as "moderate". Are there quantitative or systematic evaluation criteria for this rating?
4. In Section 2.2.4, specifically the "beta-catenin activation" part, does the discussion consider the role of its non-canonical pathways in EMT?
5. The text emphasizes the role of the Wnt/β-catenin pathway in EMT. Have other pathways with synergistic or antagonistic effects in gastric cancer been considered?
6. In Section 2.2.2, "Chronic ROS", its specific role or effects in gastric cancer models are not described.
7. For the statement in Section 2.2.5, "EMT plays an important role in therapeutic resistance and drug responses in human gastric cancer," can references providing *in vivo* evidence (e.g., from mouse models) supporting this conclusion in the context of gastric cancer be added?
8. Regarding the conclusion that this can be used for the "risk assessment of anti-cancer drugs," are there any specific application cases or examples?
9. The table in the text should be presented in a standard three-line format and should be avoided from spanning pages as much as possible.
10. Please unify the format of references in the article, including the author's name, the case of words in the title of the article, the writing of the name of the journal, and the page number.

The English could be improved to more clearly express the research.

Authors' point-to-point responses

Comments 1: This manuscript presents a well-structured and conceptually valuable Adverse Outcome Pathway (AOP) titled "Increase in Reactive Oxygen Species (ROS) and Chronic ROS Leading to Human Treatment-Resistant Gastric Cancer (GC)". The AOP delineates a mechanistic sequence from molecular initiating events (MIEs)—namely acute and sustained increases in ROS—through a series of biologically plausible key events (KEs), culminating in the adverse outcome of therapy resistant gastric cancer. But I have several following concerns:

1. In the Introduction section, the statement "The NRF2-mediated oxidative stress response network included molecules related to the Regulation of EMT by growth factors pathway and Production of nitric oxide and reactive oxygen species in macrophages, such as PI3K and AKT" should be supported by relevant literature describing the processes involved in the NRF2-mediated oxidative stress response.

Response 1: Thank you for pointing this out. We agree with this comment. Therefore, we have added the following references in the Introduction section:

1. Zhang, Q.; Liu, J.; Duan, H.; Li, R.; Peng, W.; Wu, C. Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress. J Adv Res 2021, 34, 43-63, doi:10.1016/j.jare.2021.06.023.

2. Han, X.; Zhang, Q.; Cao, D.; Wang, Y.; Wang, S.; He, Q.; Zhao, J.; Chen, X. Based on network pharmacology and experimental validation, berberine can inhibit the progression of gastric cancer by modulating oxidative stress. Transl Cancer Res 2025, 14, 554-568, doi:10.21037/tcr-24-732.

3. Hu, S.; Feng, J.; Wang, M.; Wufuer, R.; Liu, K.; Zhang, Z.; Zhang, Y. Nrf1 is an indispensable redox-determining factor for mitochondrial homeostasis by integrating multi-hierarchical regulatory networks. Redox Biol 2022, 57, 102470, doi:10.1016/j.redox.2022.102470.

Comments 2: Should the Introduction section provide references discussing the regulatory relationship between NRF2 and EMT in gastric cancer?

Response 2: We agree, and have added further references to support the regulatory relationship between NRF2 and EMT in gastric cancer: "NRF2 signaling regulated EMT in gastric cancer [4]. Additionally, EMT induction increased metastasis and cisplatin resistance in gastric cancer, which involved Nrf2 signaling [5]."

4. Guan, D.; Zhou, W.; Wei, H.; Wang, T.; Zheng, K.; Yang, C.; Feng, R.; Xu, R.; Fu, Y.; Li, C., et al. Ferritinophagy-mediated ferroptosis and activation of Keap1/Nrf2/HO-1 pathway were conducive to EMT inhibition of gastric cancer cells in action of 2,2'-di-pyridineketone hydrazone dithiocarbamate butyric acid ester. Oxid Med Cell Longev 2022, 2022, 3920664, doi:10.1155/2022/3920664.

5. Gupta, J.; Ahmed, A.T.; Tayyib, N.A.; Zabibah, R.S.; Shomurodov, Q.; Kadheim, M.N.; Alsaikhan, F.; Ramaiah, P.; Chinnasamy, L.; Samarghandian, S. A state-of-art of underlying molecular mechanisms and pharmacological interventions/nanotherapeutics for cisplatin resistance in gastric cancer. Biomed Pharmacother 2023, 166, 115337, doi:10.1016/j.biopha.2023.115337.

Comments 3: In Table 2, the "Biological Plausibility" is rated as "moderate". Are there quantitative or systematic evaluation criteria for this rating?

Response 3: Thank you very much for your essential comment. The rating of "Biological Plausibility" of Key Event Relationships (KERs) as moderate is based on the criteria "The KER is plausible based on analogy to accepted biological relationships but scientific understanding is not completely established" in the AOP guidance. The current KERs in Table 2 are rated as moderate because they are very plausible based on accepted biological relationships.

Comments 4: In Section 2.2.4, specifically the "beta-catenin activation" part, does the discussion consider the role of its non-canonical pathways in EMT?

Response 4: Thank you for your comment. "There is a possibility that non-canonical Wnt signaling, independent of beta-catenin, is involved in EMT in prostate cancer [56]. Non-canonical Wnt signatures, such as ROR2 and FZD7, are correlated with poor prognosis in gastric cancer [57]. The involvement of non-canonical pathways needs to be further investigated." (lines 222-225 in the revised manuscript.)

56. Nath, D.; Li, X.; Mondragon, C.; Post, D.; Chen, M.; White, J.R.; Hryniewicz-Jankowska, A.; Caza, T.; Kuznetsov, V.A.; Hehnly, H., et al. Abi1 loss drives prostate tumorigenesis through activation of EMT and non-canonical WNT signaling. Cell Commun Signal 2019, 17, 120, doi:10.1186/s12964-019-0410-y.

57. Astudillo, P. A Non-canonical Wnt signature correlates with lower survival in gastric cancer. Front Cell Dev Biol 2021, 9, 633675, doi:10.3389/fcell.2021.633675.

Comments 5: The text emphasizes the role of the Wnt/β-catenin pathway in EMT. Have other pathways with synergistic or antagonistic effects in gastric cancer been considered?

Response 5: Thank you very much for your comprehensive comment. Other pathways have been added in Section 3. Discussion: "The TGF-beta and SMAD signaling pathway induces EMT and gastric cancer [58], while the PI3K/AKT/mTOR signaling pathway modulates EMT and gastric cancer [59]. Hippo signaling is also implicated in gastric cancer [60]. The pathway network of gastric cancer and other cancers, with cross-talk among various signaling pathways, would be interesting to investigate in the future." (lines 225-230 in the revised manuscript.)

58. Fang, Z.; Zhang, W.; Wang, H.; Zhang, C.; Li, J.; Chen, W.; Xu, X.; Wang, L.; Ma, M.; Zhang, S., et al. Helicobacter pylori promotes gastric cancer progression by activating the TGF-β/Smad2/EMT pathway through HKDC1. Cell Mol Life Sci 2024, 81, 453, doi:10.1007/s00018-024-05491-x.

59. Fattahi, S.; Amjadi-Moheb, F.; Tabaripour, R.; Ashrafi, G.H.; Akhavan-Niaki, H. PI3K/AKT/mTOR signaling in gastric cancer: Epigenetics and beyond. Life Sci 2020, 262, 118513, doi:10.1016/j.lfs.2020.118513.

60. Cao, Z.; An, L.; Han, Y.; Jiao, S.; Zhou, Z. The Hippo signaling pathway in gastric cancer. Acta Biochim Biophys Sin (Shanghai) 2023, 55, 893-903, doi:10.3724/abbs.2023038.

Comments 6: In Section 2.2.2, "Chronic ROS", its specific role or effects in gastric cancer models are not described.

Response 6: Thank you very much for this critical comment. Section 2.2.2. Chronic ROS has been deleted as the current AOP298 has one molecular initiating event (MIE), "Increase, ROS," and the chronic ROS concept is incorporated in the key event relationship (KER).

Comments 7: For the statement in Section 2.2.5, "EMT plays an important role in therapeutic resistance and drug responses in human gastric cancer," can references providing in vivo evidence (e.g., from mouse models) supporting this conclusion in the context of gastric cancer be added?

Response 7: Thank you very much for your comprehensive suggestion. References were added to support this conclusion:

15. Zhao, Z.; Zhu, Y. FAP, CD10, and GPR77-labeled CAFs cause neoadjuvant chemotherapy resistance by inducing EMT and CSC in gastric cancer. BMC Cancer 2023, 23, 507, doi:10.1186/s12885-023-11011-0.

16. Shi, J.; Li, F.; Yao, X.; Mou, T.; Xu, Z.; Han, Z.; Chen, S.; Li, W.; Yu, J.; Qi, X., et al. The HER4-YAP1 axis promotes trastuzumab resistance in HER2-positive gastric cancer by inducing epithelial and mesenchymal transition. Oncogene 2018, 37, 3022-3038, doi:10.1038/s41388-018-0204-5.

Comments 8: Regarding the conclusion that this can be used for the "risk assessment of anti-cancer drugs," are there any specific application cases or examples?

Response 8: Thank you very much for your insightful comment. The sentence has been rephrased as follows: "the risk assessment of anti-cancer drugs, such as drug resistance prediction." (lines 237-239 in the revised manuscript.)

Comments 9: The table in the text should be presented in a standard three-line format and should be avoided from spanning pages as much as possible.

Response 9: We agree, and have accordingly modified the tables to a standard format.

Comments 10: Please unify the format of references in the article, including the author's name, the case of words in the title of the article, the writing of the name of the journal, and the page number.

Response 10: We have unified the format of references.

4. Response to Comments on the Quality of English Language

Point 1: The English could be improved to more clearly express the research.

Response 1: English was proofread by the journal's proofreading service.

Accept (Round 2)
Reviewer Comments
The author have addressed all my concerns, I recommend accepting it in current form.

Reviewer 3
Minor (Round 1)
Reviewer comments
The manuscript describes the adverse outcome pathway connecting chronic ROS accumulation, Wnt/β-catenin signaling, EMT and the development of therapy resistance in gastric cancer. Overall, the topic is quite relevant and the manuscript is clearly structured, fitting within the AOP communication format. I think the paper is suitable for publication after minor revisions.
Comments:

1. The discussion could be slightly more balanced. At the moment it focuses mainly on EMT, while other aspects of ROS-mediated tumor adaptation, like immune evasion or inflammatory feedback) could be briefly mentioned to give a more complete picture.
2. It might be good to cite some newer studies discussing the link between oxidative stress and immune modulation in gastric cancer. For example, the review https://doi.org/10.3390/ijms26031156 could be introduced.

Authors' point-to-point responses

Comments 1: The manuscript describes the adverse outcome pathway connecting chronic ROS accumulation, Wnt/β-catenin signaling, EMT and the development of therapy resistance in gastric cancer. Overall, the topic is quite relevant and the manuscript is clearly structured, fitting within the AOP communication format. I think the paper is suitable for publication after minor revisions.

1. The discussion could be slightly more balanced. At the moment it focuses mainly on EMT, while other aspects of ROS-mediated tumor adaptation, like immune evasion or inflammatory feedback could be briefly mentioned to give a more complete picture.

Response 1: Thank you for pointing this out. We agree with this comment. Therefore, we have added the discussion focusing on immune evasion and inflammation as follows:

"Oxidative stress is linked to numerous inflammatory diseases and cancer, where oxidative stress and inflammation drive the tumor cell proliferation, migration, invasion, and metastasis [52]." (lines 210-212 in the revised manuscript.)

52. Wang, M.; Xiao, Y.; Miao, J.; Zhang, X.; Liu, M.; Zhu, L.; Liu, H.; Shen, X.; Wang, J.; Xie, B., et al. Oxidative stress and inflammation: Drivers of tumorigenesis and therapeutic opportunities. Antioxidants (Basel) 2025, 14, doi:10.3390/antiox14060735.

Comments 2: It might be good to cite some newer studies discussing the link between oxidative stress and immune modulation in gastric cancer. For example, the review https://doi.org/10.3390/ijms26031156 could be introduced.

Response 2: We Agree. We have added the discussion regarding oxidative stress and immune modulation in gastric cancer in Section 3. Discussion with additional references as follows:

"The tumor microenvironment (TME), consisting of immune cells, natural killer cells, the extracellular matrix, etc., plays a critical role in tumor initiation, development, and metastasis by manipulating redox signaling [53,54]. Interactions among tumor-associated macrophages, gastric cancer cells, and natural killer cells induce immune checkpoint molecules that interact with immune cells in the TME of gastric cancer, thereby evading anti-tumor immunity. [55]. Cancer cells use chronic ROS to neutralize, exhaust, and suppress anti-tumor immune cells [53]. Persistent oxidative stress signals from cancer cells transform fibroblasts into pro-tumorigenic cancer-associated fibroblasts [53]. Chronic ROS is crucial for activating the TME in treatment-resistant cancer." (lines 213-221 in the revised manuscript.)

53. Ranbhise, J.S.; Singh, M.K.; Ju, S.; Han, S.; Yun, H.R.; Kim, S.S.; Kang, I. The redox paradox: Cancer's double-edged sword for malignancy and therapy. Antioxidants (Basel) 2025, 14, doi:10.3390/antiox14101187.

54. Arneth, B. Tumor Microenvironment. Medicina (Kaunas) 2019, 56, doi:10.3390/medicina56010015.

55. Cozac-Szőke, A.R.; Cozac, D.A.; Negovan, A.; Tinca, A.C.; Vilaia, A.; Cocuz, I.G.; Sabău, A.H.; Niculescu, R.; Chiorean, D.M.; Tomuț, A.N., et al. Immune cell interactions and immune checkpoints in the tumor microenvironment of gastric cancer. Int J Mol Sci 2025, 26, doi:10.3390/ijms26031156.