# AOP ID and Title:

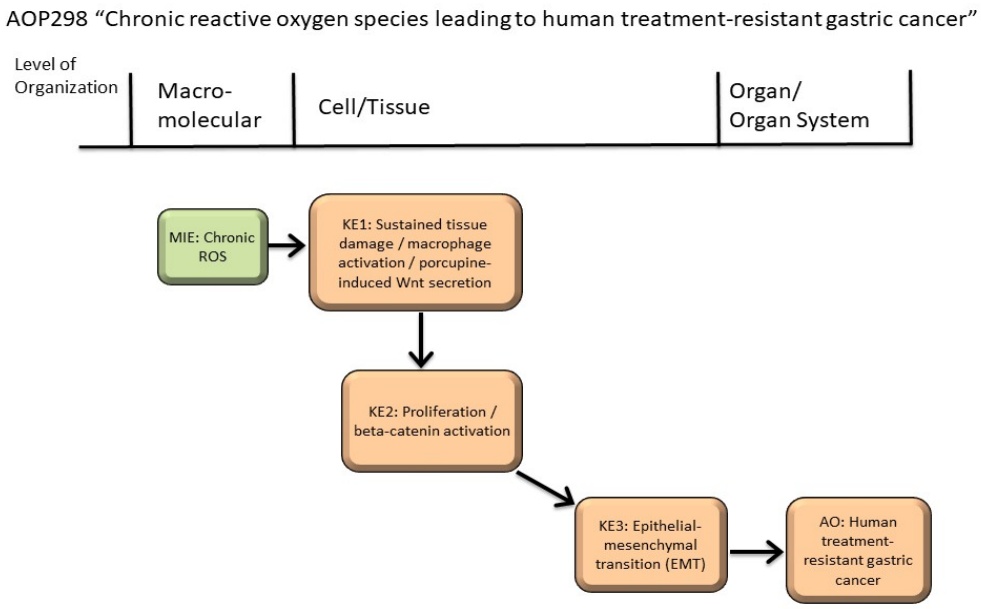
SNAPSHOT

Created at: 2020-08-06 05:41

**AOP 298: Chronic reactive oxygen species leading to human treatment-resistant gastric cancer**

**Short Title: Chronic ROS leading to human treatment-resistant gastric cancer**

# Graphical Representation




# Authors

Shihori Tanabe[1)], Sabina Quader[2)], Ryuichi Ono[3)], Horacio Cabral[4)], Kazuhiko Aoyagi[5)], Akihiko Hirose[1)], Hiroshi Yokozaki[6)], Hiroki Sasaki[7)]

[1]Division of Risk Assessment, Center for Biological Safety and Research, National Institute of Health Sciences, Japan

[2]Innovation Centre of NanoMedicine (iCONM), Kawasaki Institute of Industrial Promotion, Japan

[3]Division of Cellular and Molecular Toxicology, Center for Biological Safety and Research, National Institute of Health Sciences, Japan

[4]Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, Japan

[5]Department of Clinical Genomics, National Cancer Center Research Institute, Japan

[6]Department of Pathology, Kobe University of Graduate School of Medicine, Japan

[7]Department of Translational Oncology, National Cancer Center Research Institute, Japan

# Status

| Author status | OECD status | OECD project | SAAOP status |
|---|---|---|---|
| Under Development: Contributions and Comments Welcome | | | |

# Abstract

The injury or sustained reactive oxygen species (ROS) causes resistance in human gastric cancer. This AOP entitled “Chronic reactive oxygen species leading to human treatment-resistant gastric cancer” consists of MIE as sustained ROS, followed by KE1 as sustained tissue damage / macrophage activation / porcupine-induced Wnt secretion, KE2 as proliferation / beta-catenin activation, KE3 as epithelial-mesenchymal transition (EMT), and AO as human treatment-resistant gastric cancer. ROS has multiple roles such as development and progression of cancer, or apoptotic induction causing anti-tumor effects. In this AOP, we focus on the role of chronic ROS with sustained level to induce the therapy-resistance in human gastric cancer. EMT, which is cellular phenotypic change from epithelial to mesenchymal-like feature, demonstrates cancer stem cell-like characteristics in human gastric cancer. EMT is induced by Wnt/beta-catenin signaling, which confers rationale to have Wnt secretion and beta-catenin activation as KE1 and KE2 on the AOP, respectively.

# Summary of the AOP

## Events

### Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

| Sequence | Type | Event ID | Title | Short name |
|---|---|---|---|---|
| 1 | MIE | 1753 | Chronic reactive oxygen species (https://aopwiki.org/events/1753) | Chronic ROS |
| | | | | |
| 2 | KE | 1754 | Sustained tissue damage / macrophage activation/ porcupine-induced Wnt secretion (https://aopwiki.org/events/1754) | Sustained tissue damage / macrophage activation/ porcupine-induced Wnt secretion |
| 3 | KE | 1755 | Proliferation/ beta-catenin activation (https://aopwiki.org/events/1755) | Proliferation/ beta-catenin activation |
| 4 | KE | 1650 | Epithelial-mesenchymal transition (https://aopwiki.org/events/1650) | Epithelial-mesenchymal transition |
| | | | | |
| 5 | AO | 1651 | Treatment-resistant gastric cancer (https://aopwiki.org/events/1651) | Resistant gastric cancer |

### Key Event Relationships

| Upstream Event | Relationship Type | Downstream Event | Evidence | Quantitative Understanding |
|---|---|---|---|---|
| Chronic reactive oxygen species (https://aopwiki.org/relationships/2069) | adjacent | Sustained tissue damage / macrophage activation/ porcupine-induced Wnt secretion | Moderate | Moderate |
| Sustained tissue damage / macrophage activation/ porcupine-induced Wnt secretion (https://aopwiki.org/relationships/2070) | adjacent | Proliferation/ beta-catenin activation | High | Moderate |
| Proliferation/ beta-catenin activation (https://aopwiki.org/relationships/2071) | adjacent | Epithelial-mesenchymal transition | Moderate | Moderate |
| Epithelial-mesenchymal transition (https://aopwiki.org/relationships/1929) | adjacent | Treatment-resistant gastric cancer | High | Moderate |
| | | | | |

## Stressors

| Name | Evidence |
|---|---|
| Wnt | High |
| WNT2 | High |
| Porcupine | Moderate |
| Wntless | Moderate |
| Ionizing Radiation | Moderate |
| ferric nitrilotriacetate | Not Specified |

Wnt

WNT induces EMT (J. Zhang, Tian, & Xing, 2016).

WNT2

WNT2 induces EMT in cervical cancer (Zhou et al., 2016).

Porcupine

Porcupine palmitoleates Wnt and facilitates the secretion of the Wnt ligand (Yu & Virshup, 2014) .

Wntless

Wntless binds to and transport Wnt to the plasma membrane leading to the secretion of Wnt ligand (Yu & Virshup, 2014).

ferric nitrilotriacetate

Carcinogenic iron(III)-nitrilotriacetate induces reactive oxygen species production via trasfer of an electron to molecular oxygen to form reactive oxygen species [Tsuchiya K, Akai K, Tokumura A, Abe S, Tamaki T, Takiguchi Y, Fukuzawa K. Biochim Biophys Acta. 2005 Aug 30;1725(1):111-9. doi: 10.1016/j.bbagen.2005.05.001, Akai K, Tsuchiya K, Tokumura A, Kogure K, Ueno S, Shibata A, Tamaki T, Fukuzawa K. Free Radic Res. 2004 Sep;38(9):951-62. doi: 10.1080/10715760420002619 45.].

# Overall Assessment of the AOP

| 1. Support for Biological Plausibility of KER | |
|---|---|
| MIE => KE1:<br>Chronic ROS leads to Sustained tissue damage / macrophage activation / porcupine-induced Wnt secretion | Biological Plausibility of the MIE => KE1 is moderate.<br>Rationale: Sustained ROS increase caused by/causes DNA damage, which will alter several signaling pathways including Wnt signaling. Macrophages accumulate into 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 (Vallée & Lecarpentier, 2018).. |
| KE1 => KE2:<br>Sustained tissue damage / macrophage activation / porcupine-induced Wnt secretion leads to Proliferation / beta-catenin activation | Biological Plausibility of the KE1 => KE2 is high.<br>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 (Clevers & Nusse, 2012). |

| | |
|---|---|
| KE2 => KE3: Proliferation / beta-catenin activation leads to Epithelial-mesenchymal transition (EMT) | Biological Plausibility of the KE2 => KE3 is moderate. Rationale: Beta-catenin activation, of which mechanism include the stabilization of the dephosphorylated beta-catenin and translocation of beta-catenin into the nucleus, induce the formation of beta-catenin-TCF complex and transcription of transcription factors such as Snail, Zeb and Twist (Clevers & Nusse, 2012) (Ahmad et al., 2012; Pearlman, Montes de Oca, Pal, & Afaq, 2017; Sohn et al., 2019; W. Yang et al., 2019).<br>EMT-related transcription factors including Snail, ZEB and Twist are up-regulated in cancer cells (Diaz, Vinas-Castells, & Garcia de Herreros, 2014). 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 (Diaz et al., 2014). |
| KE3 => AO: Epithelial-mesenchymal transition (EMT) leads to human treatment-resistant gastric cancer | Biological Plausibility of the KE3 => AO is high. Rationale: Some population of the cells exhibiting EMT demonstrates the feature of cancer stem cells (CSCs), which are related to cancer malignancy (Shibue & Weinberg, 2017; Shihori Tanabe, 2015a, 2015b; Tanabe, Aoyagi, Yokozaki, & Sasaki, 2015).<br>EMT phenomenon is related to cancer metastasis and cancer therapy resistance (Smith & Bhowmick, 2016; Tanabe, 2013). Increase expression of enzymes that degrade the extracellular matrix components and the decrease in adhesion to the basement membrane in EMT induce the cell escape from the basement membrane and metastasis (Smith & Bhowmick, 2016). Morphological changes observed during EMT is associated with therapy resistance (Smith & Bhowmick, 2016). |
| 2. Support for essentiality of KEs | |
| KE1: Sustained tissue damage / macrophage activation/ porcupine-induced Wnt secretion | Essentiality of the KE1 is moderate. Rationale for Essentiality of KEs in the AOP: The sustained tissue damage, macrophage activation and Wnt are essential for the subsequent beta-catenin activation and cancer resistance. |
| KE2: Proliferation / beta-catenin activation | Essentiality of the KE2 is moderate. Rationale for Essentiality of KEs in the AOP: Proliferation and beta-catenin activation are 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 resistance to anti-cancer drug. |
| 3. Empirical support for KERs | |
| MIE => KE1: Chronic ROS leads to Sustained tissue damage / macrophage activation / porcupine-induced Wnt secretion | Empirical Support of the MIE => KE1 is moderate. Rationale: Production of ROS by DNA double-strand break causes the tissue damages (Gao et al., 2019).<br>ROS signaling induces Wnt/beta-catenin signaling (Pérez et al., 2017). |

| | |
|---|---|
| KE1 => KE2: Sustained tissue damage / macrophage activation / porcupine-induced Wnt secretion leads to Proliferation / beta-catenin activation | Empirical Support of the KE1 => KE2 is high. 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) (Clevers & Nusse, 2012; Jiang et al., 2015).<br>Wnt binds to FZD and activate the Wnt signaling (Clevers & Nusse, 2012; Janda, Waghray, Levin, Thomas, & Garcia, 2012; Nile et al., 2017). 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 (Clevers & Nusse, 2012). |
| KE2 => KE3: Proliferation / 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 (Sohn et al., 2019).<br>The garcinol, that has anti-cancer effect, increases phosphorylated beta-catenin, decreases beta-catenin and ZEB1/ZEB2, and inhibit EMT (Ahmad et al., 2012).<br>The inhibition of sortilin by AF38469 (a sortilin inhibitor) or small interference RNA (siRNA) results in decrease in beta-catenin and Twist expression in human glioblastoma cells (W. Yang et al., 2019).<br>Histone deacetylase inhibitors affect on EMT-related transcription factors including ZEB, Twist and Snail (Wawruszak et al., 2019).<br>Snail and Zeb induces EMT and suppress E-cadherin (CDH1) (Batlle et al., 2000; Diaz et al., 2014; Peinado, Olmeda, & Cano, 2007). |
| KE3 => AO: Epithelial-mesenchymal transition (EMT) leads to human treatment-resistant gastric cancer | 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 resistant to doxorubicin (Saxena, Stephens, Pathak, & Rangarajan, 2011; Shibue & Weinberg, 2017)<br>TGFbeta-1 induced EMT results in the acquisition of cancer stem cell (CSC) like properties (Pirozzi et al., 2011; Shibue & Weinberg, 2017).<br>Snail-induced EMT induces the cancer metastasis and resistance to dendritic cell-mediated immunotherapy (Kudo-Saito et al., 2009).<br>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 (Chen et al., 2014). |

## Domain of Applicability

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

*Homo sapiens*

## Essentiality of the Key Events

Sustained ROS contributes into the initiation and development of human gastric cancer (Gu H. 2018).

Wnt signaling is involved in cancer malignancy (Tanabe, 2018).

Upon stimulation with Wnt ligand to Frizzled receptor, Wnt/beta-catenin signaling is activated. Wnt/beta-catenin consists of GSK3 beta inactivation, beta-catenin activation and up-regulation of transcription factors such as Zeb, Twist and Snail. The transcription factors Zeb, Twist and Snail relate to the activation of EMT-related genes. EMT is regulated with various gene networks (Tanabe, 2015c).

## Weight of Evidence Summary

The Wnt signaling promotes EMT and cancer malignancy in colorectal cancer (Lazarova & Bordonaro, 2017). Although the potential pathways other than Wnt signaling exist in EMT induction and the mechanism underlaid cancer malignancy, Wnt signaling is one of the main pathways to induce EMT and cancer malignancy (Polakis, 2012).

## Quantitative Consideration

Wnt signaling activates the CSCs to promote cancer malignancy (Reya & Clevers, 2005). The responses in KEs related to Wnt signaling, Frizzled activation, GSK3beta inactivation, beta-catenin activation, Snail, Zeb, Twist activation are dose-dependently related. The quantification of EMT and cancer malignancy would require the further investigation.

# Considerations for Potential Applications of the AOP (optional)

AOP entitled “Chronic reactive oxygen species leading to human treatment-resistant gastric cancer” might be utilized for the development and risk assessment of anti-cancer drugs. EMT is involved in the acquisition of drug resistance, which is one of the critical features of cancer malignancy. The assessment of EMT would be the potential prediction of the adverse effects of anti-cancer drugs.

# References

Ahmad, A., Sarkar, S. H., Bitar, B., Ali, S., Aboukameel, A., Sethi, S., . . . Sarkar, F. H. (2012). Garcinol regulates EMT and Wnt signaling pathways in vitro and in vivo, leading to anticancer activity against breast cancer cells. *Mol Cancer Ther, 11*(10), 2193-2201. doi:10.1158/1535-7163.MCT-12-0232-T

Ashoka, A. H., Ali, F., Tiwari, R., Kumari, R., Pramanik, S. K., & Das, A. (2020). Recent Advances in Fluorescent Probes for Detection of HOCl and HNO. *ACS omega, 5*(4), 1730-1742. doi:10.1021/acsomega.9b03420

Banziger, C., Soldini, D., Schutt, C., Zipperlen, P., Hausmann, G., & Basler, K. (2006). Wntless, a conserved membrane protein dedicated to the secretion of Wnt proteins from signaling cells. *Cell, 125*(3), 509-522. doi:10.1016/j.cell.2006.02.049

Batlle, E., Sancho, E., Francí, C., Domínguez, D., Monfar, M., Baulida, J., & García de Herreros, A. (2000). The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nature Cell Biology, 2*(2), 84-89. doi:10.1038/35000034

Bhanot, P., Brink, M., Samos, C. H., Hsieh, J.-C., Wang, Y., Macke, J. P., . . . Nusse, R. (1996). A new member of the frizzled family from Drosophila functions as a Wingless receptor. *Nature, 382*, 225. doi:10.1038/382225a0

Bhattacharyya, A., Chattopadhyay, R., Mitra, S., & Crowe, S. E. (2014). Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiological reviews, 94*(2), 329-354. doi:10.1152/physrev.00040.2012

Bovolenta, P., Esteve, P., Ruiz, J. M., Cisneros, E., & Lopez-Rios, J. (2008). Beyond Wnt inhibition: new functions of secreted Frizzled-related proteins in development and disease. *J Cell Sci, 121*(Pt 6), 737-746. doi:10.1242/jcs.026096

Caliceti, C., Nigro, P., Rizzo, P., & Ferrari, R. (2014). ROS, Notch, and Wnt signaling pathways: crosstalk between three major regulators of cardiovascular biology. *BioMed research international, 2014*, 318714-318714. doi:10.1155/2014/318714

Cao, T. T., Xiang, D., Liu, B. L., Huang, T. X., Tan, B. B., Zeng, C. M., . . . Fu, L. (2017). FZD7 is a novel prognostic marker and promotes tumor metastasis via WNT and EMT signaling pathways in esophageal squamous cell carcinoma. *Oncotarget, 8*(39), 65957-65968. doi:10.18632/oncotarget.19586

Chen, L., Gibbons, D. L., Goswami, S., Cortez, M. A., Ahn, Y.-H., Byers, L. A., . . . Qin, F. X.-F. (2014). Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. *Nature communications, 5*, 5241-5241. doi:10.1038/ncomms6241

Cheung, E. C., Lee, P., Ceteci, F., Nixon, C., Blyth, K., Sansom, O. J., & Vousden, K. H. (2016). Opposing effects of TIGAR- and RAC1-derived ROS on Wnt-driven proliferation in the mouse intestine. *Genes & development, 30*(1), 52-63. doi:10.1101/gad.271130.115

Ching, W., & Nusse, R. (2006). A dedicated Wnt secretion factor. *Cell, 125*(3), 432-433. doi:10.1016/j.cell.2006.04.018

Clevers, H. (2006). Wnt/beta-catenin signaling in development and disease. *Cell, 127*(3), 469-480. doi:10.1016/j.cell.2006.10.018

Clevers, H., & Nusse, R. (2012). Wnt/beta-catenin signaling and disease. *Cell, 149*(6), 1192-1205. doi:10.1016/j.cell.2012.05.012

Colvin, H., Nishida, N., Konno, M., Haraguchi, N., Takahashi, H., Nishimura, J., . . . Ishii, H. (2016). Oncometabolite D-2-Hydroxyglurate Directly Induces Epithelial-Mesenchymal Transition and is Associated with Distant Metastasis in Colorectal Cancer. *Sci Rep, 6*, 36289. doi:10.1038/srep36289

Conway, J. P., & Kinter, M. (2006). Dual role of peroxiredoxin I in macrophage-derived foam cells. *The Journal of biological chemistry, 281*(38), 27991-28001. doi:10.1074/jbc.M605026200

De, A. (2011). Wnt/Ca2+ signaling pathway: a brief overview. *Acta Biochim Biophys Sin (Shanghai), 43*(10), 745-756. doi:10.1093/abbs/gmr079

Diaz, V. M., Vinas-Castells, R., & Garcia de Herreros, A. (2014). Regulation of the protein stability of EMT transcription factors. *Cell Adh Migr, 8*(4), 418-428. doi:10.4161/19336918.2014.969998

Du, B., & Shim, J. S. (2016). Targeting Epithelial-Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. *Molecules, 21*(7). doi:10.3390/molecules21070965

Du, J., Zu, Y., Li, J., Du, S., Xu, Y., Zhang, L., . . . Yang, C. (2016). Extracellular matrix stiffness dictates Wnt expression through integrin pathway. *Sci Rep, 6*, 20395. doi:10.1038/srep20395

Ellwanger, K., Saito, H., Clement-Lacroix, P., Maltry, N., Niedermeyer, J., Lee, W. K., . . . Niehrs, C. (2008). Targeted disruption of the Wnt regulator Kremen induces limb defects and high bone density. *Mol Cell Biol, 28*(15), 4875-4882. doi:10.1128/MCB.00222-08

Fang, C. X., Ma, C. M., Jiang, L., Wang, X. M., Zhang, N., Ma, J. N., . . . Zhao, Y. D. (2018). p38 MAPK is Crucial for Wnt1- and LiCl-Induced Epithelial Mesenchymal Transition. *Curr Med Sci, 38*(3), 473-481. doi:10.1007/s11596-018-1903-4

Foulquier, S., Daskalopoulos, E. P., Lluri, G., Hermans, K. C. M., Deb, A., & Blankesteijn, W. M. (2018). WNT Signaling in Cardiac and Vascular Disease. *Pharmacol Rev, 70*(1), 68-141. doi:10.1124/pr.117.013896

Funato, Y., Michiue, T., Asashima, M., & Miki, H. (2006). The thioredoxin-related redox-regulating protein nucleoredoxin inhibits Wnt–β-catenin signalling through Dishevelled. *Nature Cell Biology, 8*(5), 501-508. doi:10.1038/ncb1405

Gao, Q., Zhou, G., Lin, S.-J., Paus, R., & Yue, Z. (2019). How chemotherapy and radiotherapy damage the tissue: Comparative biology lessons from feather and hair models. *Experimental dermatology, 28*(4), 413-418. doi:10.1111/exd.13846

Gu, H., Huang, T., Shen, Y., Liu, Y., Zhou, F., Jin, Y., . . . Wei, Y. (2018). Reactive Oxygen Species-Mediated Tumor Microenvironment Transformation: The Mechanism of Radioresistant Gastric Cancer. *Oxidative medicine and cellular longevity, 2018*, 5801209-5801209. doi:10.1155/2018/5801209

Guerra, F., Guaragnella, N., Arbini, A. A., Bucci, C., Giannattasio, S., & Moro, L. (2017). Mitochondrial Dysfunction: A Novel Potential Driver of Epithelial-to-Mesenchymal Transition in Cancer. *Front Oncol, 7*, 295. doi:10.3389/fonc.2017.00295

Hatsell, S., Rowlands, T., Hiremath, M., & Cowin, P. (2003). Beta-catenin and Tcfs in mammary development and cancer. *J Mammary Gland Biol Neoplasia, 8*(2), 145-158. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14635791 (https://www.ncbi.nlm.nih.gov/pubmed/14635791)

Hodge, D. Q., Cui, J., Gamble, M. J., & Guo, W. (2018). Histone Variant MacroH2A1 Plays an Isoform-Specific Role in Suppressing Epithelial-Mesenchymal Transition. *Sci Rep, 8*(1), 841. doi:10.1038/s41598-018-19364-4

Hu, B., Cheng, J. W., Hu, J. W., Li, H., Ma, X. L., Tang, W. G., . . . Yang, X. R. (2019). KPNA3 Confers Sorafenib Resistance to Advanced Hepatocellular Carcinoma via TWIST Regulated Epithelial-Mesenchymal Transition. *Journal of Cancer, 10*(17), 3914-3925. doi:10.7150/jca.31448

Hua, Y., Yang, Y., Li, Q., He, X., Zhu, W., Wang, J., & Gan, X. (2018). Oligomerization of Frizzled and LRP5/6 protein initiates intracellular signaling for the canonical WNT/beta-catenin pathway. *J Biol Chem, 293*(51), 19710-19724. doi:10.1074/jbc.RA118.004434

Huang, J. Q., Wei, F. K., Xu, X. L., Ye, S. X., Song, J. W., Ding, P. K., . . . Gong, L. Y. (2019). SOX9 drives the epithelial-mesenchymal transition in non-small-cell lung cancer through the Wnt/beta-catenin pathway. *J Transl Med, 17*(1), 143. doi:10.1186/s12967-019-1895-2

Inukai, T., Inoue, A., Kurosawa, H., Goi, K., Shinjyo, T., Ozawa, K., . . . Look, A. T. (1999). SLUG, a ces-1-Related Zinc Finger Transcription Factor Gene with Antiapoptotic Activity, Is a Downstream Target of the E2A-HLF Oncoprotein. *Molecular Cell, 4*(3), 343-352. doi:https://doi.org/10.1016/S1097-2765(00)80336-6 (https://doi.org/10.1016/S1097-2765(00)80336-6)

Janda, C. Y., Waghray, D., Levin, A. M., Thomas, C., & Garcia, K. C. (2012). Structural basis of Wnt recognition by Frizzled. *Science, 337*(6090), 59-64. doi:10.1126/science.1222879

Jia, D., Park, J. H., Jung, K. H., Levine, H., & Kaipparettu, B. A. (2018). Elucidating the Metabolic Plasticity of Cancer: Mitochondrial Reprogramming and Hybrid Metabolic States. *Cells, 7*(3). doi:10.3390/cells7030021

Jiang, X., Charlat, O., Zamponi, R., Yang, Y., & Cong, F. (2015). Dishevelled promotes Wnt receptor degradation through recruitment of ZNRF3/RNF43 E3 ubiquitin ligases. *Mol Cell, 58*(3), 522-533. doi:10.1016/j.molcel.2015.03.015

Katoh, M. (2001). Molecular cloning and characterization of human WNT3. *Int J Oncol, 19*(5), 977-982. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11604997 (https://www.ncbi.nlm.nih.gov/pubmed/11604997)

Katoh, M. (2017). Canonical and non-canonical WNT signaling in cancer stem cells and their niches: Cellular heterogeneity, omics reprogramming, targeted therapy and tumor plasticity (Review). *International journal of oncology, 51*(5), 1357-1369. doi:10.3892/ijo.2017.4129

Kaufhold, S., & Bonavida, B. (2014). Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. *J Exp Clin Cancer Res, 33*, 62. doi:10.1186/s13046-014-0062-0

Kim, K. K., Kugler, M. C., Wolters, P. J., Robillard, L., Galvez, M. G., Brumwell, A. N., . . . Chapman, H. A. (2006). Alveolar epithelial cell mesenchymal transition develops <em>in vivo</em> during pulmonary fibrosis and is regulated by the extracellular matrix. *Proceedings of the National Academy of Sciences, 103*(35), 13180. doi:10.1073/pnas.0605669103

Kim, M., Kim, S. H., Lim, J. W., & Kim, H. (2019). Lycopene induces apoptosis by inhibiting nuclear translocation of beta-catenin in gastric cancer cells. *J Physiol Pharmacol, 70*(4). doi:10.26402/jpp.2019.4.11

Korswagen, H. C. (2006). Regulation of the Wnt/β-catenin pathway by redox signaling. *Developmental Cell, 10*(6), 687-688. doi:https://doi.org/10.1016/j.devcel.2006.05.007 (https://doi.org/10.1016/j.devcel.2006.05.007)

Kudo-Saito, C., Shirako, H., Takeuchi, T., & Kawakami, Y. (2009). Cancer Metastasis Is Accelerated through Immunosuppression during Snail-Induced EMT of Cancer Cells. *Cancer Cell, 15*(3), 195-206. doi:https://doi.org/10.1016/j.ccr.2009.01.023 (https://doi.org/10.1016/j.ccr.2009.01.023)

Kusserow, A., Pang, K., Sturm, C., Hrouda, M., Lentfer, J., Schmidt, H. A., . . . Holstein, T. W. (2005). Unexpected complexity of the Wnt gene family in a sea anemone. *Nature, 433*(7022), 156-160. doi:10.1038/nature03158

Kwon, Y. J., Baek, H. S., Ye, D. J., Shin, S., Kim, D., & Chun, Y. J. (2016). CYP1B1 Enhances Cell Proliferation and Metastasis through Induction of EMT and Activation of Wnt/beta-Catenin Signaling via Sp1 Upregulation. *PLoS One, 11*(3), e0151598. doi:10.1371/journal.pone.0151598

Lai, S. L., Chien, A. J., & Moon, R. T. (2009). Wnt/Fz signaling and the cytoskeleton: potential roles in tumorigenesis. *Cell Res, 19*(5), 532-545. doi:10.1038/cr.2009.41

Lazarova, D., & Bordonaro, M. (2017). ZEB1 Mediates Drug Resistance and EMT in p300-Deficient CRC. *Journal of Cancer, 8*(8), 1453-1459. doi:10.7150/jca.18762

Lee, D. Y., Kang, S., Lee, Y., Kim, J. Y., Yoo, D., Jung, W., . . . Jon, S. (2020). PEGylated Bilirubin-coated Iron Oxide Nanoparticles as a Biosensor for Magnetic Relaxation Switching-based ROS Detection in Whole Blood. *Theranostics, 10*(5), 1997-2007. doi:10.7150/thno.39662

Li, C., & Balazsi, G. (2018). A landscape view on the interplay between EMT and cancer metastasis. *NPJ Syst Biol Appl, 4*, 34. doi:10.1038/s41540-018-0068-x

Lin, X., Chai, G., Wu, Y., Li, J., Chen, F., Liu, J., . . . Wang, H. (2019). RNA m(6)A methylation regulates the epithelial mesenchymal transition of cancer cells and translation of Snail. *Nat Commun, 10*(1), 2065. doi:10.1038/s41467-019-09865-9

MacDonald, B. T., Tamai, K., & He, X. (2009). Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell, 17*(1), 9-26. doi:10.1016/j.devcel.2009.06.016

Mani, S. A., Guo, W., Liao, M. J., Eaton, E. N., Ayyanan, A., Zhou, A. Y., . . . Weinberg, R. A. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell, 133*(4), 704-715. doi:10.1016/j.cell.2008.03.027

Marjanovic, N. D., Weinberg, R. A., & Chaffer, C. L. (2013). Cell plasticity and heterogeneity in cancer. *Clinical chemistry, 59*(1), 168-179. doi:10.1373/clinchem.2012.184655

Menendez-Menendez, J., Hermida-Prado, F., Granda-Diaz, R., Gonzalez, A., Garcia-Pedrero, J. M., Del-Rio-Ibisate, N., . . . Martinez-Campa, C. (2019). Deciphering the Molecular Basis of Melatonin Protective Effects on Breast Cells Treated with Doxorubicin: TWIST1 a Transcription Factor Involved in EMT and Metastasis, a Novel Target of Melatonin. *Cancers (Basel), 11*(7). doi:10.3390/cancers11071011

Miller, B. A., & Cheung, J. Y. (2016). TRPM2 protects against tissue damage following oxidative stress and ischaemia-reperfusion. *The Journal of physiology, 594*(15), 4181-4191. doi:10.1113/JP270934

Mishra, P., Tang, W., Putluri, V., Dorsey, T. H., Jin, F., Wang, F., . . . Ambs, S. (2018). ADHFE1 is a breast cancer oncogene and induces metabolic reprogramming. *J Clin Invest, 128*(1), 323-340. doi:10.1172/JCI93815

Mo, M.-L., Li, M.-R., Chen, Z., Liu, X.-W., Sheng, Q., & Zhou, H.-M. (2013). Inhibition of the Wnt palmitoyltransferase porcupine suppresses cell growth and downregulates the Wnt/β-catenin pathway in gastric cancer. *Oncology letters, 5*(5), 1719-1723. doi:10.3892/ol.2013.1256

Mohammed, M. K., Shao, C., Wang, J., Wei, Q., Wang, X., Collier, Z., . . . Lee, M. J. (2016). Wnt/beta-catenin signaling plays an ever-expanding role in stem cell self-renewal, tumorigenesis and cancer chemoresistance. *Genes Dis, 3*(1), 11-40. doi:10.1016/j.gendis.2015.12.004

Myant, K. B., Cammareri, P., McGhee, E. J., Ridgway, R. A., Huels, D. J., Cordero, J. B., . . . Sansom, O. J. (2013). ROS production and NF-κB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. *Cell stem cell, 12*(6), 761-773. doi:10.1016/j.stem.2013.04.006

Naujok, O., Lentes, J., Diekmann, U., Davenport, C., & Lenzen, S. (2014). Cytotoxicity and activation of the Wnt/beta-catenin pathway in mouse embryonic stem cells treated with four GSK3 inhibitors. *BMC Res Notes, 7*, 273. doi:10.1186/1756-0500-7-273

Nile, A. H., Mukund, S., Stanger, K., Wang, W., & Hannoush, R. N. (2017). Unsaturated fatty acyl recognition by Frizzled receptors mediates dimerization upon Wnt ligand binding. *Proc Natl Acad Sci U S A, 114*(16), 4147-4152. doi:10.1073/pnas.1618293114

Ota, I., Masui, T., Kurihara, M., Yook, J. I., Mikami, S., Kimura, T., . . . Kitahara, T. (2016). Snail-induced EMT promotes cancer stem cell-like properties in head and neck cancer cells. *Oncol Rep, 35*(1), 261-266. doi:10.3892/or.2015.4348

Pearlman, R. L., Montes de Oca, M. K., Pal, H. C., & Afaq, F. (2017). Potential therapeutic targets of epithelial-mesenchymal transition in melanoma. *Cancer Lett, 391*, 125-140. doi:10.1016/j.canlet.2017.01.029

Peinado, H., Olmeda, D., & Cano, A. (2007). Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer, 7*(6), 415-428. doi:10.1038/nrc2131

Pérez, S., Taléns-Visconti, R., Rius-Pérez, S., Finamor, I., & Sastre, J. (2017). Redox signaling in the gastrointestinal tract. *Free radical biology & medicine, 104*, 75-103. doi:10.1016/j.freeradbiomed.2016.12.048

Pez, F., Lopez, A., Kim, M., Wands, J. R., Caron de Fromentel, C., & Merle, P. (2013). Wnt signaling and hepatocarcinogenesis: molecular targets for the development of innovative anticancer drugs. *J Hepatol, 59*(5), 1107-1117. doi:10.1016/j.jhep.2013.07.001

Pirozzi, G., Tirino, V., Camerlingo, R., Franco, R., La Rocca, A., Liguori, E., . . . Rocco, G. (2011). Epithelial to mesenchymal transition by TGFβ-1 induction increases stemness characteristics in primary non small cell lung cancer cell line. *PLoS One, 6*(6), e21548-e21548. doi:10.1371/journal.pone.0021548

Polakis, P. (2012). Wnt signaling in cancer. *Cold Spring Harb Perspect Biol, 4*(5). doi:10.1101/cshperspect.a008052

Qualtrough, D., Rees, P., Speight, B., Williams, A. C., & Paraskeva, C. (2015). The Hedgehog Inhibitor Cyclopamine Reduces beta-Catenin-Tcf Transcriptional Activity, Induces E-Cadherin Expression, and Reduces Invasion in Colorectal Cancer Cells. *Cancers (Basel), 7*(3), 1885-1899. doi:10.3390/cancers7030867

Reya, T., & Clevers, H. (2005). Wnt signalling in stem cells and cancer. *Nature, 434*(7035), 843-850. doi:10.1038/nature03319

Rosmaninho, P., Mükusch, S., Piscopo, V., Teixeira, V., Raposo, A. A., Warta, R., . . . Castro, D. S. (2018). Zeb1 potentiates genome-wide gene transcription with Lef1 to promote glioblastoma cell invasion. *The EMBO Journal, 37*(15), e97115. doi:10.15252/embj.201797115

Saha, S., Aranda, E., Hayakawa, Y., Bhanja, P., Atay, S., Brodin, N. P., . . . Pollard, J. W. (2016a). Macrophage-derived extracellular vesicle-packaged WNTs rescue intestinal stem cells and enhance survival after radiation injury. *Nature Communications, 7*(1), 13096. doi:10.1038/ncomms13096

Saha, S., Aranda, E., Hayakawa, Y., Bhanja, P., Atay, S., Brodin, N. P., . . . Pollard, J. W. (2016b). Macrophage-derived extracellular vesicle-packaged WNTs rescue intestinal stem cells and enhance survival after radiation injury. *Nature Communications, 7*, 13096-13096. doi:10.1038/ncomms13096

Saito-Diaz, K., Chen, T. W., Wang, X., Thorne, C. A., Wallace, H. A., Page-McCaw, A., & Lee, E. (2013). The way Wnt works: components and mechanism. *Growth Factors, 31*(1), 1-31. doi:10.3109/08977194.2012.752737

Saxena, M., Stephens, M. A., Pathak, H., & Rangarajan, A. (2011). Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. *Cell death & disease, 2*(7), e179-e179. doi:10.1038/cddis.2011.61

Sciacovelli, M., & Frezza, C. (2017). Metabolic reprogramming and epithelial-to-mesenchymal transition in cancer. *FEBS J, 284*(19), 3132-3144. doi:10.1111/febs.14090

Semenov, M. V., Zhang, X., & He, X. (2008). DKK1 antagonizes Wnt signaling without promotion of LRP6 internalization and degradation. *J Biol Chem, 283*(31), 21427-21432. doi:10.1074/jbc.M800014200

Shen, M., Bai, D., Liu, B., Lu, X., Hou, R., Zeng, C., . . . Yin, T. (2018). Dysregulated Txnip-ROS-Wnt axis contributes to the impaired ischemic heart repair in diabetic mice. *Biochimica et biophysica acta. Molecular basis of disease, 1864*(12), 3735-3745. doi:10.1016/j.bbadis.2018.09.029

Shibue, T., & Weinberg, R. A. (2017). EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol, 14*(10), 611-629. doi:10.1038/nrclinonc.2017.44

Smith, B. N., & Bhowmick, N. A. (2016). Role of EMT in Metastasis and Therapy Resistance. *J Clin Med, 5*(2). doi:10.3390/jcm5020017

Sohn, S. H., Kim, B., Sul, H. J., Kim, Y. J., Kim, H. S., Kim, H., . . . Zang, D. Y. (2019). INC280 inhibits Wnt/beta-catenin and EMT signaling pathways and its induce apoptosis in diffuse gastric cancer positive for c-MET amplification. *BMC Res Notes, 12*(1), 125. doi:10.1186/s13104-019-4163-x

Stump, B., Shrestha, S., Lamattina, A. M., Louis, P. H., Cho, W., Perrella, M. A., . . . El-Chemaly, S. (2019). Glycogen synthase kinase 3-beta inhibition induces lymphangiogenesis through beta-catenin-dependent and mTOR-independent pathways. *PLoS One, 14*(4), e0213831. doi:10.1371/journal.pone.0213831

Suarez-Carmona, M., Lesage, J., Cataldo, D., & Gilles, C. (2017). EMT and inflammation: inseparable actors of cancer progression. *Mol Oncol, 11*(7), 805-823. doi:10.1002/1878-0261.12095

Sun, J., Yang, X., Zhang, R., Liu, S., Gan, X., Xi, X., . . . Sun, Y. (2017). GOLPH3 induces epithelial-mesenchymal transition via Wnt/beta-catenin signaling pathway in epithelial ovarian cancer. *Cancer Med, 6*(4), 834-844. doi:10.1002/cam4.1040

Taelman, V. F., Dobrowolski, R., Plouhinec, J. L., Fuentealba, L. C., Vorwald, P. P., Gumper, I., . . . De Robertis, E. M. (2010). Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. *Cell, 143*(7), 1136-1148. doi:10.1016/j.cell.2010.11.034

Tanabe, S. (2013). Perspectives of gene combinations in phenotype presentation. *World journal of stem cells, 5*(3), 61-67. doi:10.4252/wjsc.v5.i3.61

Tanabe, S. (2014). Role of mesenchymal stem cells in cell life and their signaling. *World journal of stem cells, 6*(1), 24-32. doi:10.4252/wjsc.v6.i1.24

Tanabe, S. (2015a). Origin of cells and network information. *World journal of stem cells, 7*(3), 535-540. doi:10.4252/wjsc.v7.i3.535

Tanabe, S. (2015b). Signaling involved in stem cell reprogramming and differentiation. *World journal of stem cells, 7*(7), 992-998. doi:10.4252/wjsc.v7.i7.992

Tanabe, S. (2015c). Overview of gene regulation in stem cell network to identify therapeutic targets utilizing genome databases. *Insights Stem Cells, 1*(1).

Tanabe, S. (2017). Molecular markers and networks for cancer and stem cells. *J Embryol Stem Cell Res, 1*(1).

Tanabe, S. (2018). Wnt Signaling and Epithelial-Mesenchymal Transition Network in Cancer. *Res J Oncol, 2*(2).

Tanabe, S., Aoyagi, K., Yokozaki, H., & Sasaki, H. (2014). Gene expression signatures for identifying diffuse-type gastric cancer associated with epithelial-mesenchymal transition. *Int J Oncol, 44*(6), 1955-1970. doi:10.3892/ijo.2014.2387

Tanabe, S., Aoyagi, K., Yokozaki, H., & Sasaki, H. (2015). Regulated genes in mesenchymal stem cells and gastric cancer. *World journal of stem cells, 7*(1), 208-222. doi:10.4252/wjsc.v7.i1.208

Tanabe, S., Kawabata, T., Aoyagi, K., Yokozaki, H., & Sasaki, H. (2016). Gene expression and pathway analysis of CTNNB1 in cancer and stem cells. *World J Stem Cells, 8*(11), 384-395. doi:10.4252/wjsc.v8.i11.384

Tanabe, S., Komatsu, M., Kazuhiko, A., Yokozaki, H., & Sasaki, H. (2015). Implications of epithelial-mesenchymal transition in gastric cancer. *Translational Gastrointestinal Cancer, 4*(4), 258-264. Retrieved from http://tgc.amegroups.com/article/view/6996 (http://tgc.amegroups.com/article/view/6996)

Tang, Y., Shen, J., Zhang, F., Yang, F.-Y., & Liu, M. (2019). Human serum albumin attenuates global cerebral ischemia/reperfusion-induced brain injury in a Wnt/β-Catenin/ROS signaling-dependent manner in rats. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 115*, 108871-108871. doi:10.1016/j.biopha.2019.108871

Vallée, A., & Lecarpentier, Y. (2018). Crosstalk Between Peroxisome Proliferator-Activated Receptor Gamma and the Canonical WNT/β-Catenin Pathway in Chronic Inflammation and Oxidative Stress During Carcinogenesis. *Frontiers in immunology, 9*, 745-745. doi:10.3389/fimmu.2018.00745

Vikram, A., Kim, Y.-R., Kumar, S., Naqvi, A., Hoffman, T. A., Kumar, A., . . . Irani, K. (2014). Canonical Wnt signaling induces vascular endothelial dysfunction via p66Shc-regulated reactive oxygen species. *Arteriosclerosis, thrombosis, and vascular biology, 34*(10), 2301-2309. doi:10.1161/ATVBAHA.114.304338

Wang, B., Tang, Z., Gong, H., Zhu, L., & Liu, X. (2017). Wnt5a promotes epithelial-to-mesenchymal transition and metastasis in non-small-cell lung cancer. *Biosci Rep, 37*(6). doi:10.1042/BSR20171092

Wang, H. X., Li, T. Y., & Kidder, G. M. (2010). WNT2 regulates DNA synthesis in mouse granulosa cells through beta-catenin. *Biol Reprod, 82*(5), 865-875. doi:10.1095/biolreprod.109.080903

Wang, Y., Cao, P., Alshwmi, M., Jiang, N., Xiao, Z., Jiang, F., . . . Li, S. (2019). GPX2 suppression of H(2)O(2) stress regulates cervical cancer metastasis and apoptosis via activation of the β-catenin-WNT pathway. *OncoTargets and therapy, 12*, 6639-6651. doi:10.2147/OTT.S208781

Wang, Y., Shi, J., Chai, K., Ying, X., & Zhou, B. P. (2013). The Role of Snail in EMT and Tumorigenesis. *Current cancer drug targets, 13*(9), 963-972. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24168186 (https://www.ncbi.nlm.nih.gov/pubmed/24168186)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004763/ (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004763/)

Wawruszak, A., Kalafut, J., Okon, E., Czapinski, J., Halasa, M., Przybyszewska, A., . . . Stepulak, A. (2019). Histone Deacetylase Inhibitors and Phenotypical Transformation of Cancer Cells. *Cancers (Basel), 11*(2). doi:10.3390/cancers11020148

Wendt, M. K., Smith, J. A., & Schiemann, W. P. (2010). Transforming growth factor-beta-induced epithelial-mesenchymal transition facilitates epidermal growth factor-dependent breast cancer progression. *Oncogene, 29*(49), 6485-6498. doi:10.1038/onc.2010.377

Willert, K., & Nusse, R. (2012). Wnt proteins. *Cold Spring Harb Perspect Biol, 4*(9), a007864. doi:10.1101/cshperspect.a007864

Wu, W.-S., Heinrichs, S., Xu, D., Garrison, S. P., Zambetti, G. P., Adams, J. M., & Look, A. T. (2005). Slug Antagonizes p53-Mediated Apoptosis of Hematopoietic Progenitors by Repressing puma. *Cell, 123*(4), 641-653. doi:https://doi.org/10.1016/j.cell.2005.09.029 (https://doi.org/10.1016/j.cell.2005.09.029)

Xue, Y., Zhang, L., Zhu, Y., Ke, X., Wang, Q., & Min, H. (2019). Regulation of Proliferation and Epithelial-to-Mesenchymal Transition (EMT) of Gastric Cancer by ZEB1 via Modulating Wnt5a and Related Mechanisms. *Medical science monitor : international medical journal of experimental and clinical research, 25*, 1663-1670. doi:10.12659/MSM.912338

Yang, K. T., Chang, W. L., Yang, P. C., Chien, C. L., Lai, M. S., Su, M. J., & Wu, M. L. (2006). Activation of the transient receptor potential M2 channel and poly(ADP-ribose) polymerase is involved in oxidative stress-induced cardiomyocyte death. *Cell Death & Differentiation, 13*(10), 1815-1826. doi:10.1038/sj.cdd.4401813

Yang, W., Wu, P. F., Ma, J. X., Liao, M. J., Wang, X. H., Xu, L. S., . . . Yi, L. (2019). Sortilin promotes glioblastoma invasion and mesenchymal transition through GSK-3beta/beta-catenin/twist pathway. *Cell Death Dis, 10*(3), 208. doi:10.1038/s41419-019-1449-9

Yu, J., & Virshup, David M. (2014). Updating the Wnt pathways. *Bioscience Reports, 34*(5). doi:10.1042/BSR20140119

Zeisberg, M., & Neilson, E. G. (2009). Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest, 119*(6), 1429-1437. doi:10.1172/JCI36183

Zeng, H., Lu, B., Zamponi, R., Yang, Z., Wetzel, K., Loureiro, J., . . . Cong, F. (2018). mTORC1 signaling suppresses Wnt/beta-catenin signaling through DVL-dependent regulation of Wnt receptor FZD level. *Proc Natl Acad Sci U S A, 115*(44), E10362-E10369. doi:10.1073/pnas.1808575115

Zhang, J., Tian, X. J., & Xing, J. (2016). Signal Transduction Pathways of EMT Induced by TGF-beta, SHH, and WNT and Their Crosstalks. *J Clin Med, 5*(4). doi:10.3390/jcm5040041

Zhang, P., Sun, Y., & Ma, L. (2015). ZEB1: at the crossroads of epithelial-mesenchymal transition, metastasis and therapy resistance. *Cell Cycle, 14*(4), 481-487. doi:10.1080/15384101.2015.1006048

Zhang, Z., Wang, X., Cheng, S., Sun, L., Son, Y.-O., Yao, H., . . . Shi, X. (2011). Reactive oxygen species mediate arsenic induced cell transformation and tumorigenesis through Wnt/β-catenin pathway in human colorectal adenocarcinoma DLD1 cells. *Toxicology and Applied Pharmacology, 256*(2), 114-121. doi:https://doi.org/10.1016/j.taap.2011.07.016 (https://doi.org/10.1016/j.taap.2011.07.016)

Zhou, Y., Huang, Y., Cao, X., Xu, J., Zhang, L., Wang, J., . . . Zheng, M. (2016). WNT2 Promotes Cervical Carcinoma Metastasis and Induction of Epithelial-Mesenchymal Transition. *PLoS One, 11*(8), e0160414. doi:10.1371/journal.pone.0160414

Ziv, E., Yarmohammadi, H., Boas, F. E., Petre, E. N., Brown, K. T., Solomon, S. B., . . . Erinjeri, J. P. (2017). Gene Signature Associated with Upregulation of the Wnt/beta-Catenin Signaling Pathway Predicts Tumor Response to Transarterial Embolization. *J Vasc Interv Radiol, 28*(3), 349-355 e341. doi:10.1016/j.jvir.2016.11.004

# Appendix 1

## List of MIEs in this AOP

Event: 1753: Chronic reactive oxygen species (https://aopwiki.org/events/1753)

Short Name: Chronic ROS

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:298 - Chronic reactive oxygen species leading to human treatment-resistant gastric cancer (https://aopwiki.org/aops/298) | MolecularInitiatingEvent |

### Stressors

| Name |
|---|
| Ionizing Radiation |
| ferric nitrilotriacetate |

### Biological Context

| Level of Biological Organization |
| --- |
| Molecular |

## Evidence for Perturbation by Stressor

### Ionizing Radiation

Ionizing radiation induces reactive oxygen species.

(Ref. Reactive Oxygen and Nitrogen Species in Carcinogenesis: Implications of Oxidative Stress on the Progression and Development of Several Cancer Types

Author(s): Joanna Kruk, Hassan Y. Aboul-Enein*. Journal Name: Mini-Reviews in Medicinal Chemistry,

Volume 17 , Issue 11 , 2017, DOI : 10.2174/1389557517666170228115324)

### ferric nitrilotriacetate

Iron(III)-nitrilotriacetate induces reactive oxygen species production via trasfer of an electron to molecular oxygen to form reactive oxygen species [Tsuchiya K, Akai K, Tokumura A, Abe S, Tamaki T, Takiguchi Y, Fukuzawa K. Biochim Biophys Acta. 2005 Aug 30;1725(1):111-9. doi: 10.1016/j.bbagen.2005.05.001, Akai K, Tsuchiya K, Tokumura A, Kogure K, Ueno S, Shibata A, Tamaki T, Fukuzawa K. Free Radic Res. 2004 Sep;38(9):951-62. doi: 10.1080/1071576042000261945.]

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
| --- | --- | --- | --- |
| Homo sapiens | Homo sapiens | Moderate | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
| --- | --- |
| All life stages | Moderate |

**Sex Applicability**

| Sex | Evidence |
| --- | --- |
| Unspecific | High |

ROS is increased in human gastric cancer (*Homo sapiens*) (Gu et al., 2018).

### Key Event Description

Site of action: The site of action for the molecular initiating event is DNA or proteins.

Reactive oxygen species (ROS) play an important role in tumorigenesis (Zhang et al., 2011).

ROS is generated through NADPH oxidases consists of p47phox and p67phox. Arsenic produces ROS (Zhang et al., 2011).

Chronic low-level increased ROS can alter the tumor microenvironment and promote cancer stem cell renewal, leading to therapeutic resistance (Gu et al., 2018).

### How it is Measured or Detected

ROS in blood can be detected using superparamagnetic iron oxide nanoparticles (SPION)-based biosensor (Lee et al., 2020).

ROS can be detected by fluorescent probes such as *p*-methoxy-phenol derivative (Ashoka et al., 2020).

### References

Ashoka, A. H., Ali, F., Tiwari, R., Kumari, R., Pramanik, S. K., & Das, A. (2020). Recent Advances in Fluorescent Probes for Detection of HOCl and HNO. *ACS omega, 5*(4), 1730-1742. doi:10.1021/acsomega.9b03420

Gu, H., Huang, T., Shen, Y., Liu, Y., Zhou, F., Jin, Y., . . . Wei, Y. (2018). Reactive Oxygen Species-Mediated Tumor Microenvironment Transformation: The Mechanism of Radioresistant Gastric Cancer. *Oxidative medicine and cellular longevity, 2018*, 5801209-5801209. doi:10.1155/2018/5801209

Lee, D. Y., Kang, S., Lee, Y., Kim, J. Y., Yoo, D., Jung, W., . . . Jon, S. (2020). PEGylated Bilirubin-coated Iron Oxide Nanoparticles as a Biosensor for Magnetic Relaxation Switching-based ROS Detection in Whole Blood. *Theranostics, 10*(5), 1997-2007. doi:10.7150/thno.39662

Zhang, Z., Wang, X., Cheng, S., Sun, L., Son, Y.-O., Yao, H., . . . Shi, X. (2011). Reactive oxygen species mediate arsenic induced cell transformation and tumorigenesis through Wnt/β-catenin pathway in human colorectal adenocarcinoma DLD1 cells. *Toxicology and Applied Pharmacology, 256*(2), 114-121. doi:https://doi.org/10.1016/j.taap.2011.07.016 (https://doi.org/10.1016/j.taap.2011.07.016)

## List of Key Events in the AOP

### Event: 1754: Sustained tissue damage / macrophage activation/ porcupine-induced Wnt secretion (https://aopwiki.org/events/1754)

Short Name: Sustained tissue damage / macrophage activation/ porcupine-induced Wnt secretion

#### Key Event Component

| Process | Object | Action |
|---|---|---|
| Wnt protein secretion | protein-serine O-palmitoleoyltransferase porcupine | increased |

#### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:298 - Chronic reactive oxygen species leading to human treatment-resistant gastric cancer (https://aopwiki.org/aops/298) | KeyEvent |

#### Stressors

| Name |
|---|
| Radiation |

#### Biological Context

| Level of Biological Organization |
|---|
| Tissue |

## Evidence for Perturbation by Stressor

#### Radiation

Radiation induces porcupine-induced Wnt secretion in macrophage (Saha et al., 2016a).

#### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | Moderate |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

Oligomerization of FZD and low-density lipoprotein receptor-related protein 5/6 (LRP5/6) activates Wnt/beta-catenin signaling in *Homo sapiens* (Hua et al., 2018).

## Key Event Description

Porcupine, which is a trans-membrane endoplasmic reticulum O-acyl transferase, which is important for the secretion of Wnt ligands(Saha et al., 2016a). WNTs are secreted proteins that contain 22-24 conserved cysteine residues (Foulquier et al., 2018). The WNT molecules consist of molecular families including WNT1, WNT2, WNT2B/WNT13, WNT3, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT10B, WNT11 and WNT16. (Clevers & Nusse, 2012; M. Katoh, 2001; Kusserow et al., 2005)

Wnt proteins consists of 350-400 amino acids (Saito-Diaz et al., 2013).

WNT ligands are known to trigger at least three different downstream signaling cascades including canonical WNT/beta-catenin signaling pathway, non-canonical WNT/$Ca^{2+}$ pathway and planer cell polarity (PCP) pathway(De, 2011; Lai, Chien, & Moon, 2009; Willert & Nusse, 2012). WNTs bind to Frizzled proteins, which are seven-pass transmembrane receptors with an extracellular N-terminal cysteine-rich domain (Bhanot et al., 1996; Clevers, 2006). Wnt signaling begins with the binding of Wnt ligand towards the Frizzled receptors (Mohammed et al., 2016).

Wnt ligands bind to Frizzled (FZD) receptors which are seven transmembrane-domain protein receptors (Nile, Mukund, Stanger, Wang, & Hannoush, 2017). At least 10 FZD receptors are identified in human cells. FZD receptor is activated by Wnt ligand binding (MacDonald, Tamai, & He, 2009).

## How it is Measured or Detected

- Secretion of WNT requires a number of other dedicated factors including the sortin receptor Wntless (WLS), which binds to Wnt and escorts it to the cell surface (Banziger et al., 2006; Ching & Nusse, 2006)
- Wnt signaling is activated by the gene mutations of the signaling components (Ziv et al., 2017).
- Wnt1, Wnt3a and Wnt5a protein expression are measured by immunoblotting using antibodies for Wnt1, Wnt3a and Wnt5a, respectively (J. Du et al., 2016; B. Wang et al., 2017).
- WNT2, of which expression is detected by quantitative PCR, immunoblotting and immunohistochemistry, induces EMT (Zhou et al., 2016).
- Frizzled receptor protein level on the cell surface is measured by flow cytometry with pan-FZD antibody (Jiang et al., 2015; Zeng et al., 2018). DVL protein level is measured by immunoblotting with anti-DVL2 antibody (Zeng et al., 2018).
- Fzd mRNA level is measured by quantitative reverse transcription-polymerase chain reaction (RT-PCR) (Zeng et al., 2018).
- The up-regulation of WNT ligand expression occurs in *Homo sapiens* (B. Wang et al., 2017).
- The Wnt genes play an important roles in the secretion from cells, glycosylation and tight association with the cell surface and extracellular matrix in *Drosophila melanogaster* (Willert & Nusse, 2012).

## References


Banziger, C., Soldini, D., Schutt, C., Zipperlen, P., Hausmann, G., & Basler, K. (2006). Wntless, a conserved membrane protein dedicated to the secretion of Wnt proteins from signaling cells. *Cell, 125*(3), 509-522. doi:10.1016/j.cell.2006.02.049

Bhanot, P., Brink, M., Samos, C. H., Hsieh, J.-C., Wang, Y., Macke, J. P., . . . Nusse, R. (1996). A new member of the frizzled family from Drosophila functions as a Wingless receptor. *Nature, 382*, 225. doi:10.1038/382225a0

Ching, W., & Nusse, R. (2006). A dedicated Wnt secretion factor. *Cell, 125*(3), 432-433. doi:10.1016/j.cell.2006.04.018

Clevers, H. (2006). Wnt/beta-catenin signaling in development and disease. *Cell, 127*(3), 469-480. doi:10.1016/j.cell.2006.10.018

Clevers, H., & Nusse, R. (2012). Wnt/beta-catenin signaling and disease. *Cell, 149*(6), 1192-1205. doi:10.1016/j.cell.2012.05.012

De, A. (2011). Wnt/Ca2+ signaling pathway: a brief overview. *Acta Biochim Biophys Sin (Shanghai), 43*(10), 745-756. doi:10.1093/abbs/gmr079

Du, J., Zu, Y., Li, J., Du, S., Xu, Y., Zhang, L., . . . Yang, C. (2016). Extracellular matrix stiffness dictates Wnt expression through integrin pathway. *Sci Rep, 6*, 20395. doi:10.1038/srep20395

Foulquier, S., Daskalopoulos, E. P., Lluri, G., Hermans, K. C. M., Deb, A., & Blankesteijn, W. M. (2018). WNT Signaling in Cardiac and Vascular Disease. *Pharmacol Rev, 70*(1), 68-141. doi:10.1124/pr.117.013896

Hua, Y., Yang, Y., Li, Q., He, X., Zhu, W., Wang, J., & Gan, X. (2018). Oligomerization of Frizzled and LRP5/6 protein initiates intracellular signaling for the canonical WNT/beta-catenin pathway. *J Biol Chem, 293*(51), 19710-19724. doi:10.1074/jbc.RA118.004434

Jiang, X., Charlat, O., Zamponi, R., Yang, Y., & Cong, F. (2015). Dishevelled promotes Wnt receptor degradation through recruitment of ZNRF3/RNF43 E3 ubiquitin ligases. *Mol Cell, 58*(3), 522-533. doi:10.1016/j.molcel.2015.03.015

Katoh, M. (2001). Molecular cloning and characterization of human WNT3. *International journal of oncology, 19*(5), 977-982. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11604997 (https://www.ncbi.nlm.nih.gov/pubmed/11604997)

Kusserow, A., Pang, K., Sturm, C., Hrouda, M., Lentfer, J., Schmidt, H. A., . . . Holstein, T. W. (2005). Unexpected complexity of the Wnt gene family in a sea anemone. *Nature, 433*(7022), 156-160. doi:10.1038/nature03158

Lai, S. L., Chien, A. J., & Moon, R. T. (2009). Wnt/Fz signaling and the cytoskeleton: potential roles in tumorigenesis. *Cell Res, 19*(5), 532-545. doi:10.1038/cr.2009.41

MacDonald, B. T., Tamai, K., & He, X. (2009). Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell, 17*(1), 9-26. doi:10.1016/j.devcel.2009.06.016

Mohammed, M. K., Shao, C., Wang, J., Wei, Q., Wang, X., Collier, Z., . . . Lee, M. J. (2016). Wnt/beta-catenin signaling plays an ever-expanding role in stem cell self-renewal, tumorigenesis and cancer chemoresistance. *Genes Dis, 3*(1), 11-40. doi:10.1016/j.gendis.2015.12.004

Nile, A. H., Mukund, S., Stanger, K., Wang, W., & Hannoush, R. N. (2017). Unsaturated fatty acyl recognition by Frizzled receptors mediates dimerization upon Wnt ligand binding. *Proc Natl Acad Sci U S A, 114*(16), 4147-4152. doi:10.1073/pnas.1618293114

Saha, S., Aranda, E., Hayakawa, Y., Bhanja, P., Atay, S., Brodin, N. P., . . . Pollard, J. W. (2016). Macrophage-derived extracellular vesicle-packaged WNTs rescue intestinal stem cells and enhance survival after radiation injury. *Nature Communications, 7*(1), 13096. doi:10.1038/ncomms13096

Saito-Diaz, K., Chen, T. W., Wang, X., Thorne, C. A., Wallace, H. A., Page-McCaw, A., & Lee, E. (2013). The way Wnt works: components and mechanism. *Growth Factors, 31*(1), 1-31. doi:10.3109/08977194.2012.752737

Wang, B., Tang, Z., Gong, H., Zhu, L., & Liu, X. (2017). Wnt5a promotes epithelial-to-mesenchymal transition and metastasis in non-small-cell lung cancer. *Biosci Rep, 37*(6). doi:10.1042/BSR20171092

Willert, K., & Nusse, R. (2012). Wnt proteins. *Cold Spring Harb Perspect Biol, 4*(9), a007864. doi:10.1101/cshperspect.a007864

Zeng, H., Lu, B., Zamponi, R., Yang, Z., Wetzel, K., Loureiro, J., . . . Cong, F. (2018). mTORC1 signaling suppresses Wnt/beta-catenin signaling through DVL-dependent regulation of Wnt receptor FZD level. *Proc Natl Acad Sci U S A, 115*(44), E10362-E10369. doi:10.1073/pnas.1808575115

Zhou, Y., Huang, Y., Cao, X., Xu, J., Zhang, L., Wang, J., . . . Zheng, M. (2016). WNT2 Promotes Cervical Carcinoma Metastasis and Induction of Epithelial-Mesenchymal Transition. *PLoS One, 11*(8), e0160414. doi:10.1371/journal.pone.0160414

Ziv, E., Yarmohammadi, H., Boas, F. E., Petre, E. N., Brown, K. T., Solomon, S. B., . . . Erinjeri, J. P. (2017). Gene Signature Associated with Upregulation of the Wnt/beta-Catenin Signaling Pathway Predicts Tumor Response to Transarterial Embolization. *J Vasc Interv Radiol, 28*(3), 349-355 e341. doi:10.1016/j.jvir.2016.11.004

## Event: 1755: Proliferation/ beta-catenin activation (https://aopwiki.org/events/1755)

Short Name: Proliferation/ beta-catenin activation

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:298 - Chronic reactive oxygen species leading to human treatment-resistant gastric cancer (https://aopwiki.org/aops/298) | KeyEvent |

### Biological Context

| Level of Biological Organization |
|---|
| Cellular |

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | Moderate |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

Beta-catenin is stabilized and translocated into nucleus in *Homo sapiens* (Huang et al., 2019).

Beta-catenin is activated in *Homo sapiens* (Huang et al., 2019) (Naujok et al., 2014).

## Key Event Description

Upon the Wnt signaling activation, beta-catenin is stabilized and activated via inhibition of the phosphorylation by GSK3beta (Huang et al., 2019). Once the beta-catenin is stabilized, it translocates into the nucleus and enhance the expression of target genes of Wnt/beta-catenin signaling pathway (Huang et al., 2019). Beta-catenin activation is related to cancer(Tanabe, 2014).

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) (Clevers & Nusse, 2012 (https://aopwiki.org/events/1754#_ENREF_14); Jiang, et al., 2015 (https://aopwiki.org/events/1754#_ENREF_36)). DVL, however, has a controversial role to promote Wnt receptor degradation (Jiang et al., 2015 (https://aopwiki.org/events/1754#_ENREF_36)). Meanwhile, DVL-dependent regulation of FZD level is involved in mTORC1 signaling suppression via Wnt/beta-catenin signaling (Zeng et al., 2018 (https://aopwiki.org/events/1754#_ENREF_113)).

## How it is Measured or Detected

The beta-catenin level in nucleus is measured by immunoblotting with anti-beta-catenin antibody (Huang et al., 2019).

The beta-catenin nuclear translocation is measured by immunofluorescence assay (Huang et al., 2019).

Activity of beta-catenin is measured by Wnt/beta-catenin activity assay, in which the vector containing the firefly luciferase gene controlled by TCF/LEF binding sites is transfected in the cells (Naujok et al., 2014).

## References


Clevers, H., & Nusse, R. (2012). Wnt/beta-catenin signaling and disease. *Cell, 149*(6), 1192-1205. doi:10.1016/j.cell.2012.05.012

Huang, J. Q., Wei, F. K., Xu, X. L., Ye, S. X., Song, J. W., Ding, P. K., . . . Gong, L. Y. (2019). SOX9 drives the epithelial-mesenchymal transition in non-small-cell lung cancer through the Wnt/beta-catenin pathway. *J Transl Med, 17*(1), 143. doi:10.1186/s12967-019-1895-2

Jiang, X., Charlat, O., Zamponi, R., Yang, Y., & Cong, F. (2015). Dishevelled promotes Wnt receptor degradation through recruitment of ZNRF3/RNF43 E3 ubiquitin ligases. *Mol Cell, 58*(3), 522-533. doi:10.1016/j.molcel.2015.03.015

Naujok, O., Lentes, J., Diekmann, U., Davenport, C., & Lenzen, S. (2014). Cytotoxicity and activation of the Wnt/beta-catenin pathway in mouse embryonic stem cells treated with four GSK3 inhibitors. *BMC Res Notes, 7*, 273. doi:10.1186/1756-0500-7-273

Tanabe, S. (2014). Role of mesenchymal stem cells in cell life and their signaling. *World journal of stem cells, 6*(1), 24-32. doi:10.4252/wjsc.v6.i1.24

Zeng, H., Lu, B., Zamponi, R., Yang, Z., Wetzel, K., Loureiro, J., . . . Cong, F. (2018). mTORC1 signaling suppresses Wnt/beta-catenin signaling through DVL-dependent regulation of Wnt receptor FZD level. *Proc Natl Acad Sci U S A, 115*(44), E10362-E10369. doi:10.1073/pnas.1808575115


## Event: 1650: Epithelial-mesenchymal transition (https://aopwiki.org/events/1650)

Short Name: Epithelial-mesenchymal transition

## AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:298 - Chronic reactive oxygen species leading to human treatment-resistant gastric cancer (https://aopwiki.org/aops/298) | KeyEvent |

## Stressors

| Name |
|---|
| GOLPH3 |
| LiCl |
| D-2-hydroxyglutarate |

### Biological Context

| Level of Biological Organization |
|---|
| Cellular |

### Cell term

| Cell term |
|---|
| cell |

### Organ term

| Organ term |
|---|
| organ |

## Evidence for Perturbation by Stressor

### GOLPH3

GOLPH3 induces EMT (Sun et al., 2017).

### LiCl

LiCl induces EMT (Fang et al., 2018).

### D-2-hydroxyglutarate

D-2-hydroxyglutarate induces EMT (Colvin et al., 2016).

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

- Wnt5a expression leads to epithelial-mesenchymal transition (EMT) and metastasis in non-small-cell lung cancer in *Homo sapiens* (Wang et al., 2017).
- WNT2 expression lead to EMT induction in *Homo sapiens* (Zhou et al., 2016).
- EMT is induced in cancer and involved in cancer metastasis in *Homo sapiens* (Suarez-Carmona, Lesage, Cataldo, & Gilles, 2017) (Du & Shim, 2016).

## Key Event Description

Epithelial-mesenchymal transition (EMT) is a phenomenon in which the cells transit from epithelial-like into mesenchymal-like phenotypes (S. Tanabe, 2017; Shihori Tanabe, Komatsu, Kazuhiko, Yokozaki, & Sasaki, 2015). In cancer, cells exhibiting EMT features contribute into the metastasis and drug resistance.

It is known that D-2-hydroxyglurate induces EMT(Guerra et al., 2017; Jia, Park, Jung, Levine, & Kaipparettu, 2018; Mishra et al., 2018; Sciacovelli & Frezza, 2017). D-2-hydroxyglurate, an inhibitor of Jumonji-family histone demethylase, increased the trimethylation of histone H3 lysine 4 (H3K4) in the promoter region of the ZEB1, followed by the induction of EMT (Colvin et al., 2016).

Wnt5a induces EMT and metastasis in non-small-cell lung cancer (Wang, Tang, Gong, Zhu, & Liu, 2017).

EMT is related to Wnt/beta-catenin signaling and important for cancer (S. Tanabe, Kawabata, Aoyagi, Yokozaki, & Sasaki, 2016)

TGFb induces EMT (Wendt, Smith, & Schiemann, 2010).

ZEB is one of the important transcription factors for EMT regulation (Zhang, Sun, & Ma, 2015).

SNAI1 (Snail) is an important transcription factor for cell differentiation and survival, and the phosphorylation and nuclear localization of Snail1 induced by Wnt signaling pathways are critical for the regulation of EMT (Kaufhold & Bonavida, 2014).

Transcription factors SNAI1 and TWIST1 induce EMT (Hodge, Cui, Gamble, & Guo, 2018) (Mani et al., 2008)

It is suggested that Sp1, a transcription factor involved in cell growth and metastasis, is induced by cytochrome P450 1B1 (CYP1B1), and promotes EMT, which leads to cell proliferation and metastasis (Kwon et al., 2016).

## How it is Measured or Detected

- EMT can be detected by immunostaining with pro-surfactant protein-C (pro-SPC) and N-cadherin in idiopathic pulmonary fibrosis (IPF) lung *in vivo* (Kim et al., 2006).
- TGFbeta induces EMT, which can be detected by immunostaining with vimentin in lung aloevela *in vivo* (Kim et al., 2006).

## References


Colvin, H., Nishida, N., Konno, M., Haraguchi, N., Takahashi, H., Nishimura, J., . . . Ishii, H. (2016). Oncometabolite D-2-Hydroxyglurate Directly Induces Epithelial-Mesenchymal Transition and is Associated with Distant Metastasis in Colorectal Cancer. *Sci Rep, 6*, 36289. doi:10.1038/srep36289

Du, B., & Shim, J. S. (2016). Targeting Epithelial-Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. *Molecules, 21*(7). doi:10.3390/molecules21070965

Fang, C. X., Ma, C. M., Jiang, L., Wang, X. M., Zhang, N., Ma, J. N., . . . Zhao, Y. D. (2018). p38 MAPK is Crucial for Wnt1- and LiCl-Induced Epithelial Mesenchymal Transition. *Curr Med Sci, 38*(3), 473-481. doi:10.1007/s11596-018-1903-4

Guerra, F., Guaragnella, N., Arbini, A. A., Bucci, C., Giannattasio, S., & Moro, L. (2017). Mitochondrial Dysfunction: A Novel Potential Driver of Epithelial-to-Mesenchymal Transition in Cancer. *Front Oncol, 7*, 295. doi:10.3389/fonc.2017.00295

Hodge, D. Q., Cui, J., Gamble, M. J., & Guo, W. (2018). Histone Variant MacroH2A1 Plays an Isoform-Specific Role in Suppressing Epithelial-Mesenchymal Transition. *Sci Rep, 8*(1), 841. doi:10.1038/s41598-018-19364-4

Jia, D., Park, J. H., Jung, K. H., Levine, H., & Kaipparettu, B. A. (2018). Elucidating the Metabolic Plasticity of Cancer: Mitochondrial Reprogramming and Hybrid Metabolic States. *Cells, 7*(3). doi:10.3390/cells7030021

Kaufhold, S., & Bonavida, B. (2014). Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. *J Exp Clin Cancer Res, 33*, 62. doi:10.1186/s13046-014-0062-0

Kim, K. K., Kugler, M. C., Wolters, P. J., Robillard, L., Galvez, M. G., Brumwell, A. N., . . . Chapman, H. A. (2006). Alveolar epithelial cell mesenchymal transition develops <em>in vivo</em> during pulmonary fibrosis and is regulated by the extracellular matrix. *Proceedings of the National Academy of Sciences, 103*(35), 13180. doi:10.1073/pnas.0605669103

Kwon, Y. J., Baek, H. S., Ye, D. J., Shin, S., Kim, D., & Chun, Y. J. (2016). CYP1B1 Enhances Cell Proliferation and Metastasis through Induction of EMT and Activation of Wnt/beta-Catenin Signaling via Sp1 Upregulation. *PLoS One, 11*(3), e0151598. doi:10.1371/journal.pone.0151598

Mani, S. A., Guo, W., Liao, M. J., Eaton, E. N., Ayyanan, A., Zhou, A. Y., . . . Weinberg, R. A. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell, 133*(4), 704-715. doi:10.1016/j.cell.2008.03.027

Mishra, P., Tang, W., Putluri, V., Dorsey, T. H., Jin, F., Wang, F., . . . Ambs, S. (2018). ADHFE1 is a breast cancer oncogene and induces metabolic reprogramming. *J Clin Invest, 128*(1), 323-340. doi:10.1172/JCI93815

Sciacovelli, M., & Frezza, C. (2017). Metabolic reprogramming and epithelial-to-mesenchymal transition in cancer. *FEBS J, 284*(19), 3132-3144. doi:10.1111/febs.14090

Suarez-Carmona, M., Lesage, J., Cataldo, D., & Gilles, C. (2017). EMT and inflammation: inseparable actors of cancer progression. *Mol Oncol, 11*(7), 805-823. doi:10.1002/1878-0261.12095

Sun, J., Yang, X., Zhang, R., Liu, S., Gan, X., Xi, X., . . . Sun, Y. (2017). GOLPH3 induces epithelial-mesenchymal transition via Wnt/beta-catenin signaling pathway in epithelial ovarian cancer. *Cancer Med, 6*(4), 834-844. doi:10.1002/cam4.1040

Tanabe, S. (2017). Molecular markers and networks for cancer and stem cells. *J Embryol Stem Cell Res, 1*(1).

Tanabe, S., Kawabata, T., Aoyagi, K., Yokozaki, H., & Sasaki, H. (2016). Gene expression and pathway analysis of CTNNB1 in cancer and stem cells. *World J Stem Cells, 8*(11), 384-395. doi:10.4252/wjsc.v8.i11.384

Tanabe, S., Komatsu, M., Kazuhiko, A., Yokozaki, H., & Sasaki, H. (2015). Implications of epithelial-mesenchymal transition in gastric cancer. *Translational Gastrointestinal Cancer, 4*(4), 258-264. Retrieved from http://tgc.amegroups.com/article/view/6996 (http://tgc.amegroups.com/article/view/6996)

Wang, B., Tang, Z., Gong, H., Zhu, L., & Liu, X. (2017). Wnt5a promotes epithelial-to-mesenchymal transition and metastasis in non-small-cell lung cancer. *Biosci Rep, 37*(6). doi:10.1042/BSR20171092

Wendt, M. K., Smith, J. A., & Schiemann, W. P. (2010). Transforming growth factor-beta-induced epithelial-mesenchymal transition facilitates epidermal growth factor-dependent breast cancer progression. *Oncogene, 29*(49), 6485-6498. doi:10.1038/onc.2010.377

Zhang, P., Sun, Y., & Ma, L. (2015). ZEB1: at the crossroads of epithelial-mesenchymal transition, metastasis and therapy resistance. *Cell Cycle, 14*(4), 481-487. doi:10.1080/15384101.2015.1006048

Zhou, Y., Huang, Y., Cao, X., Xu, J., Zhang, L., Wang, J., . . . Zheng, M. (2016). WNT2 Promotes Cervical Carcinoma Metastasis and Induction of Epithelial-Mesenchymal Transition. *PLoS One, 11*(8), e0160414. doi:10.1371/journal.pone.0160414

## List of Adverse Outcomes in this AOP

Event: 1651: Treatment-resistant gastric cancer (https://aopwiki.org/events/1651)

Short Name: Resistant gastric cancer

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:298 - Chronic reactive oxygen species leading to human treatment-resistant gastric cancer (https://aopwiki.org/aops/298) | AdverseOutcome |

### Biological Context

| Level of Biological Organization |
|---|
| Tissue |

### Organ term

| Organ term |
|---|
| organ |

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

Drug resistance occurs in *Homo sapiens* (Du & Shim, 2016).

### Key Event Description

Drug resistance is involved in EMT, which is an important phenomenon exhibiting the feature similar to cancer stem cells (CSCs) (Du & Shim, 2016).

EMT is involved in metastasis and therapy resistance (Smith & Bhowmick, 2016).

Diffuse-type gastric cancer which has a poor prognosis may be related to EMT (Tanabe, Aoyagi, Yokozaki, & Sasaki, 2014).

### How it is Measured or Detected

Cancer malignancy and EMT can be detected with biomarkers (Zeisberg & Neilson, 2009).

EMT can be detected as the increase level of the transcription factors, Zeb, Twist and Snail, related to the activation of EMT-related genes.

### Regulatory Significance of the AO

Cancer resistance is very important in the cancer treatment, since the cancer metastasis and recurrence are one of the main obstacles to treat cancer.

### References

Du, B., & Shim, J. S. (2016). Targeting Epithelial-Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. *Molecules, 21*(7). doi:10.3390/molecules21070965

Smith, B. N., & Bhowmick, N. A. (2016). Role of EMT in Metastasis and Therapy Resistance. *J Clin Med, 5*(2). doi:10.3390/jcm5020017

Tanabe, S., Aoyagi, K., Yokozaki, H., & Sasaki, H. (2014). Gene expression signatures for identifying diffuse-type gastric cancer associated with epithelial-mesenchymal transition. *International journal of oncology, 44*(6), 1955-1970. doi:10.3892/ijo.2014.2387

Zeisberg, M., & Neilson, E. G. (2009). Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest, 119*(6), 1429-1437. doi:10.1172/JCI36183

# Appendix 2

# List of Key Event Relationships in the AOP

## List of Adjacent Key Event Relationships

Relationship: 2069: Chronic ROS leads to Sustained tissue damage / macrophage activation/ porcupine-induced Wnt secretion (https://aopwiki.org/relationships/2069)

AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Chronic reactive oxygen species leading to human treatment-resistant gastric cancer (https://aopwiki.org/aops/298)** | **adjacent** | **Moderate** | **Moderate** |

Key Event Relationship Description

ROS production causes the tissue damage (Gao, Zhou, Lin, Paus, & Yue, 2019).. ROS production is involved in Wnt-driven tumorigenesis (Myant et al., 2013).

Injury causes the Porcupine-induced Wnt secretion (Saha et al., 2016).

## Evidence Supporting this KER

**Biological Plausibility**

Sustained ROS increase caused by/causes DNA damage, which will alter several signaling pathways including Wnt signaling. Macrophages accumulate into 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 (Vallée & Lecarpentier, 2018).

**Empirical Evidence**

Production of ROS by DNA double-strand break causes the tissue damages (Gao et al., 2019).

ROS signaling induces Wnt/beta-catenin signaling(Pérez, Taléns-Visconti, Rius-Pérez, Finamor, & Sastre, 2017).

**Uncertainties and Inconsistencies**

The balance of ROS signaling is important, and dual effects of ROS should be taken in consideration. The ROS may enhance Wnt/beta-catenin proliferating pathways to promote tumorigenesis, while ROS may disrupt tumor progression by different pro-apoptotic mechanisms (Pérez et al., 2017). It is also known that Wnt signaling induces ROS signaling (Cheung et al., 2016). Wnt/beta-catenin signaling control by ROS needs to be further investigated (Caliceti, Nigro, Rizzo, & Ferrari, 2014).

## Quantitative Understanding of the Linkage

**Response-response relationship**

ROS induces inflammatory responses (Bhattacharyya, Chattopadhyay, Mitra, & Crowe, 2014). Oxidant induces ROS generation and p38 MAPK activation in macrophages (Conway & Kinter, 2006). ROS induce tissue damage in cardiac myocytes (Miller & Cheung, 2016; Yang et al., 2006).

**Time-scale**

For the colony formation assay, cells were treated with 400 microM/L $H_2O_2$ for 1 week, where the medium was changed every three days (Wang et al., 2019).

**Known modulating factors**

GPX2, an activator of Wnt/beta-catenin signaling, is identified as a key regulator of intracellular $H_2O_2$ levels and an inhibitor of apoptosis (Wang et al., 2019).

**Known Feedforward/Feedback loops influencing this KER**

The reduction in ROS levels in the human serum albumin-treated cerebral ischemia/reperfusion-induced injury may be mediated by Wnt/beta-catenin signaling (Tang, Shen, Zhang, Yang, & Liu, 2019).

## References


Bhattacharyya, A., Chattopadhyay, R., Mitra, S., & Crowe, S. E. (2014). Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiological reviews, 94*(2), 329-354. doi:10.1152/physrev.00040.2012

Caliceti, C., Nigro, P., Rizzo, P., & Ferrari, R. (2014). ROS, Notch, and Wnt signaling pathways: crosstalk between three major regulators of cardiovascular biology. *BioMed research international, 2014*, 318714-318714. doi:10.1155/2014/318714

Cheung, E. C., Lee, P., Ceteci, F., Nixon, C., Blyth, K., Sansom, O. J., & Vousden, K. H. (2016). Opposing effects of TIGAR- and RAC1-derived ROS on Wnt-driven proliferation in the mouse intestine. *Genes & development, 30*(1), 52-63. doi:10.1101/gad.271130.115

Conway, J. P., & Kinter, M. (2006). Dual role of peroxiredoxin I in macrophage-derived foam cells. *The Journal of biological chemistry, 281*(38), 27991-28001. doi:10.1074/jbc.M605026200

Gao, Q., Zhou, G., Lin, S.-J., Paus, R., & Yue, Z. (2019). How chemotherapy and radiotherapy damage the tissue: Comparative biology lessons from feather and hair models. *Experimental dermatology, 28*(4), 413-418. doi:10.1111/exd.13846

Miller, B. A., & Cheung, J. Y. (2016). TRPM2 protects against tissue damage following oxidative stress and ischaemia-reperfusion. *The Journal of physiology, 594*(15), 4181-4191. doi:10.1113/JP270934

Myant, K. B., Cammareri, P., McGhee, E. J., Ridgway, R. A., Huels, D. J., Cordero, J. B., . . . Sansom, O. J. (2013). ROS production and NF-κB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. *Cell stem cell, 12*(6), 761-773. doi:10.1016/j.stem.2013.04.006

Pérez, S., Taléns-Visconti, R., Rius-Pérez, S., Finamor, I., & Sastre, J. (2017). Redox signaling in the gastrointestinal tract. *Free radical biology & medicine, 104*, 75-103. doi:10.1016/j.freeradbiomed.2016.12.048

Saha, S., Aranda, E., Hayakawa, Y., Bhanja, P., Atay, S., Brodin, N. P., . . . Pollard, J. W. (2016). Macrophage-derived extracellular vesicle-packaged WNTs rescue intestinal stem cells and enhance survival after radiation injury. *Nature Communications, 7*, 13096-13096. doi:10.1038/ncomms13096

Tang, Y., Shen, J., Zhang, F., Yang, F.-Y., & Liu, M. (2019). Human serum albumin attenuates global cerebral ischemia/reperfusion-induced brain injury in a Wnt/β-Catenin/ROS signaling-dependent manner in rats. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 115*, 108871-108871. doi:10.1016/j.biopha.2019.108871

Vallée, A., & Lecarpentier, Y. (2018). Crosstalk Between Peroxisome Proliferator-Activated Receptor Gamma and the Canonical WNT/β-Catenin Pathway in Chronic Inflammation and Oxidative Stress During Carcinogenesis. *Frontiers in immunology, 9*, 745-745. doi:10.3389/fimmu.2018.00745

Wang, Y., Cao, P., Alshwmi, M., Jiang, N., Xiao, Z., Jiang, F., . . . Li, S. (2019). GPX2 suppression of H(2)O(2) stress regulates cervical cancer metastasis and apoptosis via activation of the β-catenin-WNT pathway. *OncoTargets and therapy, 12*, 6639-6651. doi:10.2147/OTT.S208781

Yang, K. T., Chang, W. L., Yang, P. C., Chien, C. L., Lai, M. S., Su, M. J., & Wu, M. L. (2006). Activation of the transient receptor potential M2 channel and poly(ADP-ribose) polymerase is involved in oxidative stress-induced cardiomyocyte death. *Cell Death & Differentiation, 13*(10), 1815-1826. doi:10.1038/sj.cdd.4401813

## Relationship: 2070: Sustained tissue damage / macrophage activation/ porcupine-induced Wnt secretion leads to Proliferation/ beta-catenin activation (https://aopwiki.org/relationships/2070)

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Chronic reactive oxygen species leading to human treatment-resistant gastric cancer (https://aopwiki.org/aops/298)** | **adjacent** | **High** | **Moderate** |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

GSK3-beta inhibition induced beta-catenin activation in human lung lymphatic endothelial cells (*Homo sapiens*) (Stump et al., 2019).

### Key Event Relationship Description

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 (Clevers & Nusse, 2012).

### Evidence Supporting this KER

**Biological Plausibility**

Canonical Wnt pathway consists of Wnt, GSK3beta and beta-catenin cascade (Clevers & Nusse, 2012; Hatsell, Rowlands, Hiremath, & Cowin, 2003).

GSK3beta recruitment to LRP6 leads to form un-phosphorylated beta-catenin inducing the stabilization and translocation of the beta-catenin (MacDonald, Tamai, & He, 2009).

Stabilized beta-catenin accumulates in cytosol and translocates into the nucleus leading to beta-catenin activation (MacDonald et al., 2009).

**Empirical Evidence**

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) (Clevers & Nusse, 2012; Jiang, Charlat, Zamponi, Yang, & Cong, 2015).

Wnt binds to FZD and activate the Wnt signaling (Clevers & Nusse, 2012; Janda, Waghray, Levin, Thomas, & Garcia, 2012; Nile, Mukund, Stanger, Wang, & Hannoush, 2017). 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 (Clevers & Nusse, 2012).

**Uncertainties and Inconsistencies**

Some Wnt ligands bind to FZD, leading to Wnt/beta-catenin signaling inactivation. DVL, a positive regulator of Wnt signaling, has a controversial role to promote Wnt receptor degradation (Jiang et al., 2015). DVL-dependent regulation of FZD level is involved in mTORC1 signaling suppression via Wnt/beta-catenin signaling (Zeng et al., 2018)

GSK3beta phosphorylates LRP6 as well as remaining GSK3 beta phosphorylates beta-catenin which would be ubiquitinated and degradated (MacDonald et al., 2009).

## Quantitative Understanding of the Linkage

**Response-response relationship**

Wnt3 promotes proliferation and survival in HUVECs (Shen et al., 2018).

GSK3beta inhibition by 1 uM of SB216763 or 5 uM of BRD3731 results in the decreased phosphorylation and stabilization of beta-catenin (Stump et al., 2019). The level of beta-catenin is increased by the inhibition of GSK3beta kinase activity (Stump et al., 2019). GSK3beta inhibition by small interference RNA (siRNA) of GSK3beta results in the decreased phosphorylation and increased expression of beta-catenin (Stump et al., 2019).

**Time-scale**

FZD7 enhances the activity of canonical Wnt/beta-catenin signaling with the treatment of WNT3A for 1 to 6 hrs (Cao et al., 2017). The treatment with SB216763 or BRD3731, GSK3beta inhibitors, decreases phosphorylated beta-catenin and increased beta-catenin expression in 48 hours (Stump et al., 2019). The cells are treated with GSK3beta small interference RNA (siRNA) for 48 hours to silence the expression of GSK3beta, which results in the activation of beta-catenin pathway (Stump et al., 2019).

**Known modulating factors**

FZD5 can activate WNT3A/beta-catenin signaling in a dose-dependent manner (Hua et al., 2018). The increase in FZD5 protein enhances cell response to WNT3A. (Hua et al., 2018). LRP5 can augment WNT3A/beta-catenin signaling in a dose-dependent manner (Hua et al., 2018). The binding of Wnt and FZD induce the formation of the protein complex with the Dvl, Axin, CK1 GSK3, beta-catenin and APC to induce the beta-catenin translocation into the nucleus (Clevers & Nusse, 2012).

**Known Feedforward/Feedback loops influencing this KER**

Beta-catenin is required and sufficient for the sequestration of GSK3 in acidic cytoplasmic endosomes (Taelman et al., 2010). Beta-catenin, of which level increases in Wnt signaling, facilitates GSK3 sequestration leading to feed-forward loop formation (Taelman et al., 2010). The Wnt ligand is antagonized with secreted Frizzled-related proteins (sFRPs) and Wnt inhibitory protein (WIF), both of which can bind Wnts and inhibit interactions between WNT and FZD (Bovolenta, Esteve, Ruiz, Cisneros, & Lopez-Rios, 2008; Clevers & Nusse, 2012). The Dickkopf 1 (DKK1) can disrupts Wnt-induced FZD-LRP6 complex formation (Clevers & Nusse, 2012; Ellwanger et al., 2008; Semenov, Zhang, & He, 2008).

## References


Bovolenta, P., Esteve, P., Ruiz, J. M., Cisneros, E., & Lopez-Rios, J. (2008). Beyond Wnt inhibition: new functions of secreted Frizzled-related proteins in development and disease. *J Cell Sci, 121*(Pt 6), 737-746. doi:10.1242/jcs.026096

Cao, T. T., Xiang, D., Liu, B. L., Huang, T. X., Tan, B. B., Zeng, C. M., . . . Fu, L. (2017). FZD7 is a novel prognostic marker and promotes tumor metastasis via WNT and EMT signaling pathways in esophageal squamous cell carcinoma. *Oncotarget, 8*(39), 65957-65968. doi:10.18632/oncotarget.19586

Clevers, H., & Nusse, R. (2012). Wnt/beta-catenin signaling and disease. *Cell, 149*(6), 1192-1205. doi:10.1016/j.cell.2012.05.012

Ellwanger, K., Saito, H., Clement-Lacroix, P., Maltry, N., Niedermeyer, J., Lee, W. K., . . . Niehrs, C. (2008). Targeted disruption of the Wnt regulator Kremen induces limb defects and high bone density. *Mol Cell Biol, 28*(15), 4875-4882. doi:10.1128/MCB.00222-08

Hatsell, S., Rowlands, T., Hiremath, M., & Cowin, P. (2003). Beta-catenin and Tcfs in mammary development and cancer. *J Mammary Gland Biol Neoplasia, 8*(2), 145-158. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14635791 (https://www.ncbi.nlm.nih.gov/pubmed/14635791)

Hua, Y., Yang, Y., Li, Q., He, X., Zhu, W., Wang, J., & Gan, X. (2018). Oligomerization of Frizzled and LRP5/6 protein initiates intracellular signaling for the canonical WNT/beta-catenin pathway. *J Biol Chem, 293*(51), 19710-19724. doi:10.1074/jbc.RA118.004434

Janda, C. Y., Waghray, D., Levin, A. M., Thomas, C., & Garcia, K. C. (2012). Structural basis of Wnt recognition by Frizzled. *Science, 337*(6090), 59-64. doi:10.1126/science.1222879

Jiang, X., Charlat, O., Zamponi, R., Yang, Y., & Cong, F. (2015). Dishevelled promotes Wnt receptor degradation through recruitment of ZNRF3/RNF43 E3 ubiquitin ligases. *Mol Cell, 58*(3), 522-533. doi:10.1016/j.molcel.2015.03.015

MacDonald, B. T., Tamai, K., & He, X. (2009). Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell, 17*(1), 9-26. doi:10.1016/j.devcel.2009.06.016

Nile, A. H., Mukund, S., Stanger, K., Wang, W., & Hannoush, R. N. (2017). Unsaturated fatty acyl recognition by Frizzled receptors mediates dimerization upon Wnt ligand binding. *Proc Natl Acad Sci U S A, 114*(16), 4147-4152. doi:10.1073/pnas.1618293114

Semenov, M. V., Zhang, X., & He, X. (2008). DKK1 antagonizes Wnt signaling without promotion of LRP6 internalization and degradation. *J Biol Chem, 283*(31), 21427-21432. doi:10.1074/jbc.M800014200

Shen, M., Bai, D., Liu, B., Lu, X., Hou, R., Zeng, C., . . . Yin, T. (2018). Dysregulated Txnip-ROS-Wnt axis contributes to the impaired ischemic heart repair in diabetic mice. *Biochimica et biophysica acta. Molecular basis of disease, 1864*(12), 3735-3745. doi:10.1016/j.bbadis.2018.09.029

Stump, B., Shrestha, S., Lamattina, A. M., Louis, P. H., Cho, W., Perrella, M. A., . . . El-Chemaly, S. (2019). Glycogen synthase kinase 3-beta inhibition induces lymphangiogenesis through beta-catenin-dependent and mTOR-independent pathways. *PLoS One, 14*(4), e0213831. doi:10.1371/journal.pone.0213831

Taelman, V. F., Dobrowolski, R., Plouhinec, J. L., Fuentealba, L. C., Vorwald, P. P., Gumper, I., . . . De Robertis, E. M. (2010). Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. *Cell, 143*(7), 1136-1148. doi:10.1016/j.cell.2010.11.034

Zeng, H., Lu, B., Zamponi, R., Yang, Z., Wetzel, K., Loureiro, J., . . . Cong, F. (2018). mTORC1 signaling suppresses Wnt/beta-catenin signaling through DVL-dependent regulation of Wnt receptor FZD level. *Proc Natl Acad Sci U S A, 115*(44), E10362-E10369. doi:10.1073/pnas.1808575115

## Relationship: 2071: Proliferation/ beta-catenin activation leads to Epithelial-mesenchymal transition (https://aopwiki.org/relationships/2071)

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Chronic reactive oxygen species leading to human treatment-resistant gastric cancer (https://aopwiki.org/aops/298)** | **adjacent** | **Moderate** | **Moderate** |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

- The inhibition of c-MET decreases the expression of beta-catenin and Snail in human diffuse-type gastric cancer (*Homo sapiens*) (Sohn et al., 2019).

The treatment with garcinol decreases the expression of beta-catenin and ZEB1/ZEB2 in human breast cancer cells (*Homo sapiens*) (Ahmad et al., 2012).

Zeb1 activation leads to EMT via Prex1 activation in NCH421k, NCH441, and NCH644 human glioblastoma model cells (*Homo sapien*s) (Rosmaninho et al., 2018).

Zeb1 siRNA induced the suppression of EMT in SGC-7901 human gastric cancer cell line (*Homo sapiens*) (Xue et al., 2019). Snail induces EMT in SAS and HSC-4 human head and neck squamous cancer cells (*Homo sapiens*) (Ota et al., 2016).

Snail induces EMT in B16-F10 murine melanoma cells (*Mus musculus*) (Kudo-Saito, Shirako, Takeuchi, & Kawakami, 2009; Wang, Shi, Chai, Ying, & Zhou, 2013).

Twist1 is related to EMT in MCF-7 and MDA-MB-231 human breast cancer cell lines (*Homo sapiens*) (Menendez-Menendez et al., 2019). Twist induces EMT in Huh7 human hepatocellular carcinoma cell lines (*Homo sapiens*) (Hu et al., 2019).

### Key Event Relationship Description

Beta-catenin activation, of which mechanism include the stabilization of the dephosphorylated beta-catenin and translocation of beta-catenin into the nucleus, induce the formation of beta-catenin-TCF complex and transcription of transcription factors such as Snail, Zeb and Twist (Clevers & Nusse, 2012) (Ahmad et al., 2012; Pearlman, Montes de Oca, Pal, & Afaq, 2017; Sohn et al., 2019; Yang et al., 2019).

EMT-related transcription factors including Snail, ZEB and Twist are up-regulated in cancer cells (Diaz, Vinas-Castells, & Garcia de Herreros, 2014). 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 (Diaz et al., 2014).

## Evidence Supporting this KER

**Biological Plausibility**

The treatment of human gastric cancer cells with INC280, which inhibits c-MET overexpressed in diffuse-type gastric cancer with poor prognosis, shows downregulation in beta-catenin and Snail expression,(Sohn et al., 2019).

The treatment with garcinol, a polyisoprenylated benzophenone derivative that is obtained from *Garcinia indica* extract, induced ZEB1 and ZEB2 down-regulation, increase in phosphorylated beta-catenin and decrease in nuclear beta-catenin in human breast cancer cells (Ahmad et al., 2012).

Sortilin, a member of the Vps10p sorting receptor family which is highly expressed in high-glade malignant glioma, positively regulates GSK-3beta/beta-catenin/Twist signaling pathway in glioblastoma (Yang et al., 2019).

The transcription factors such as Snail, Zeb and Twist inhibit the CDH1 expression through their binding towards the promoter of CDH1, which leads to inhibition of cell adhesion and EMT (Diaz et al., 2014)

**Empirical Evidence**

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 (Sohn et al., 2019).

The garcinol, that has anti-cancer effect, increases phosphorylated beta-catenin, decreases beta-catenin and ZEB1/ZEB2, and inhibit EMT (Ahmad et al., 2012).

The inhibition of sortilin by AF38469 (a sortilin inhibitor) or small interference RNA (siRNA) results in decrease in beta-catenin and Twist expression in human glioblastoma cells (Yang et al., 2019).

Histone deacetylase inhibitors affect on EMT-related transcription factors including ZEB, Twist and Snail (Wawruszak et al., 2019).

Snail and Zeb induces EMT and suppress E-cadherin (CDH1) (Batlle et al., 2000; Diaz et al., 2014; Peinado, Olmeda, & Cano, 2007).

**Uncertainties and Inconsistencies**

It is possible that the inhibition of ZEB1 and ZEB2 by garcinol treatment is caused by down-regulation of NFkappaB and Wnt/beta catenin signaling (Ahmad et al., 2012).

The EMT is induced different transcription factors other than Zeb, Twist and Snail, which includes E47 and KLF8 (Diaz et al., 2014).

Zeb, Twist and Snail may activate or inactivate different genes or molecules to induce phenomena related to EMT and other phenomena other than EMT (Li & Balazsi, 2018).

## Quantitative Understanding of the Linkage

**Response-response relationship**

The treatment with AF38469, a sortilin inhibitor, in 0, 100, 200, 400, 800, and 1600 nM concentration inhibited beta-catenin and Twist expression dose-dependently in human glioblastoma cells (Yang et al., 2019).

Snail (SNAI1) mRNA is methylated and $N^6$-methyladenosine ($m^6A$) in its coding region (CDS) and 3' untranslated region (3'UTR) are significantly enriched during EMT progression (Lin et al., 2019). The $m^6A$ enrichment fold of *SNAI1* mRNA in EMT cells is about 2.3-fold greater than in control cells (Lin et al., 2019).

**Time-scale**

The treatment with 25 uM of garcinol for 48 hours induced increase in phosphorylated beta-catenin and decreased nuclear beta-catenin protein and ZEB1/ZEB2 mRNA in human breast cancer cells (Ahmad et al., 2012).

The treatment with AF38469, a sortilin inhibitor, for 0, 2, 4, 8, 16, or 24 hours shows that the expression of beta-catenin and Twist decrease in 8 hours followed by the subsequent decrease in 16 and 24 hours in human gliobastoma cells (Yang et al., 2019).

Snail (SNAI1) transfection for 48 hours induce the repression of E-cadherin (CDH1) protein expression (Lin et al., 2019).

SNAI1 mRNA in polysome is up-regulated in EMT-undergoing HeLa cells treated with 10 ng/ml of TGF-beta for 3 days compared with control cells (Lin et al., 2019).

**Known modulating factors**

The proto-oncogene MET regulates beta-catenin and Snail expression (Sohn et al., 2019).

The inhibition of GSK3beta by SB216763 induced expression of beta-catenin and Twist, as well as mesenchymal markers such as N-cadherin, vimentin and MMP9 (Yang et al., 2019).

The decrease in E-cadherin (CDH1), a cell adhesion molecule, is related to EMT (Diaz et al., 2014).

Methyltransferase-like 3 (METTL3) modulates methylation of Snail (SNAI1) mRNA and EMT (Lin et al., 2019).

Binding of beta-catenin to members of the TCF/LEF family transcription factors increase gene expression related to EMT such as Twist and decrease E-cadherin protein expression (Qualtrough, Rees, Speight, Williams, & Paraskeva, 2015).

**Known Feedforward/Feedback loops influencing this KER**

The inhibited expression of phosphorylated GSK3beta, beta-catenin and Twist by sortilin inhibition is reversed by GSK3beta inhibition. Furthermore, twist overexpression by lentivirus increased the inhibited expression of N-cadherin, MMP9 and vimentin and reverses the inhibitory effect of AF38469 on sortilin, which suggests that sortilin induces glioblastoma invasion mainly via GSK3beta/beta-catenin/Twist induced mesenchymal transition (Yang et al., 2019).

The inhibition of Hedgehog signaling pathway with cyclopamine reduces beta-catenin-TCF transcriptional activity, decreases the Twist expression, induces E-cadherin expression and inhibits EMT (Qualtrough et al., 2015).

## References


Ahmad, A., Sarkar, S. H., Bitar, B., Ali, S., Aboukameel, A., Sethi, S., . . . Sarkar, F. H. (2012). Garcinol regulates EMT and Wnt signaling pathways in vitro and in vivo, leading to anticancer activity against breast cancer cells. *Mol Cancer Ther, 11*(10), 2193-2201. doi:10.1158/1535-7163.MCT-12-0232-T

Batlle, E., Sancho, E., Francí, C., Domínguez, D., Monfar, M., Baulida, J., & García de Herreros, A. (2000). The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nature Cell Biology, 2*(2), 84-89. doi:10.1038/35000034

Clevers, H., & Nusse, R. (2012). Wnt/beta-catenin signaling and disease. *Cell, 149*(6), 1192-1205. doi:10.1016/j.cell.2012.05.012

Diaz, V. M., Vinas-Castells, R., & Garcia de Herreros, A. (2014). Regulation of the protein stability of EMT transcription factors. *Cell Adh Migr, 8*(4), 418-428. doi:10.4161/19336918.2014.969998

Hu, B., Cheng, J. W., Hu, J. W., Li, H., Ma, X. L., Tang, W. G., . . . Yang, X. R. (2019). KPNA3 Confers Sorafenib Resistance to Advanced Hepatocellular Carcinoma via TWIST Regulated Epithelial-Mesenchymal Transition. *Journal of Cancer, 10*(17), 3914-3925. doi:10.7150/jca.31448

Kudo-Saito, C., Shirako, H., Takeuchi, T., & Kawakami, Y. (2009). Cancer Metastasis Is Accelerated through Immunosuppression during Snail-Induced EMT of Cancer Cells. *Cancer Cell, 15*(3), 195-206. doi:https://doi.org/10.1016/j.ccr.2009.01.023 (https://doi.org/10.1016/j.ccr.2009.01.023)

Li, C., & Balazsi, G. (2018). A landscape view on the interplay between EMT and cancer metastasis. *NPJ Syst Biol Appl, 4*, 34. doi:10.1038/s41540-018-0068-x

Lin, X., Chai, G., Wu, Y., Li, J., Chen, F., Liu, J., . . . Wang, H. (2019). RNA m(6)A methylation regulates the epithelial mesenchymal transition of cancer cells and translation of Snail. *Nat Commun, 10*(1), 2065. doi:10.1038/s41467-019-09865-9

Menendez-Menendez, J., Hermida-Prado, F., Granda-Diaz, R., Gonzalez, A., Garcia-Pedrero, J. M., Del-Rio-Ibisate, N., . . . Martinez-Campa, C. (2019). Deciphering the Molecular Basis of Melatonin Protective Effects on Breast Cells Treated with Doxorubicin: TWIST1 a Transcription Factor Involved in EMT and Metastasis, a Novel Target of Melatonin. *Cancers (Basel), 11*(7). doi:10.3390/cancers11071011

Ota, I., Masui, T., Kurihara, M., Yook, J. I., Mikami, S., Kimura, T., . . . Kitahara, T. (2016). Snail-induced EMT promotes cancer stem cell-like properties in head and neck cancer cells. *Oncol Rep, 35*(1), 261-266. doi:10.3892/or.2015.4348

Pearlman, R. L., Montes de Oca, M. K., Pal, H. C., & Afaq, F. (2017). Potential therapeutic targets of epithelial-mesenchymal transition in melanoma. *Cancer Lett, 391*, 125-140. doi:10.1016/j.canlet.2017.01.029

Peinado, H., Olmeda, D., & Cano, A. (2007). Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer, 7*(6), 415-428. doi:10.1038/nrc2131

Qualtrough, D., Rees, P., Speight, B., Williams, A. C., & Paraskeva, C. (2015). The Hedgehog Inhibitor Cyclopamine Reduces beta-Catenin-Tcf Transcriptional Activity, Induces E-Cadherin Expression, and Reduces Invasion in Colorectal Cancer Cells. *Cancers (Basel), 7*(3), 1885-1899. doi:10.3390/cancers7030867

Rosmaninho, P., Mükusch, S., Piscopo, V., Teixeira, V., Raposo, A. A., Warta, R., . . . Castro, D. S. (2018). Zeb1 potentiates genome-wide gene transcription with Lef1 to promote glioblastoma cell invasion. *The EMBO Journal, 37*(15), e97115. doi:10.15252/embj.201797115

Sohn, S. H., Kim, B., Sul, H. J., Kim, Y. J., Kim, H. S., Kim, H., . . . Zang, D. Y. (2019). INC280 inhibits Wnt/beta-catenin and EMT signaling pathways and its induce apoptosis in diffuse gastric cancer positive for c-MET amplification. *BMC Res Notes, 12*(1), 125. doi:10.1186/s13104-019-4163-x

Wang, Y., Shi, J., Chai, K., Ying, X., & Zhou, B. P. (2013). The Role of Snail in EMT and Tumorigenesis. *Current cancer drug targets, 13*(9), 963-972. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24168186 (https://www.ncbi.nlm.nih.gov/pubmed/24168186)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004763/ (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004763/)

Wawruszak, A., Kalafut, J., Okon, E., Czapinski, J., Halasa, M., Przybyszewska, A., . . . Stepulak, A. (2019). Histone Deacetylase Inhibitors and Phenotypical Transformation of Cancer Cells. *Cancers (Basel), 11*(2). doi:10.3390/cancers11020148

Xue, Y., Zhang, L., Zhu, Y., Ke, X., Wang, Q., & Min, H. (2019). Regulation of Proliferation and Epithelial-to-Mesenchymal Transition (EMT) of Gastric Cancer by ZEB1 via Modulating Wnt5a and Related Mechanisms. *Medical science monitor : international medical journal of experimental and clinical research, 25*, 1663-1670. doi:10.12659/MSM.912338

Yang, W., Wu, P. F., Ma, J. X., Liao, M. J., Wang, X. H., Xu, L. S., . . . Yi, L. (2019). Sortilin promotes glioblastoma invasion and mesenchymal transition through GSK-3beta/beta-catenin/twist pathway. *Cell Death Dis, 10*(3), 208. doi:10.1038/s41419-019-1449-9

Relationship: 1929: Epithelial-mesenchymal transition leads to Resistant gastric cancer (https://aopwiki.org/relationships/1929)

AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Chronic reactive oxygen species leading to human treatment-resistant gastric cancer (https://aopwiki.org/aops/298)** | **adjacent** | **High** | **Moderate** |

Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

EMT induces cancer invasion, metastasis (*Homo sapiens*)(P. Zhang et al., 2015).

EMT is related to cancer drug resistance in MCF-7 human breast cancer cells (*Homo sapiens*)(B. Du & Shim, 2016).

## Key Event Relationship Description

Some population of the cells exhibiting EMT demonstrates the feature of cancer stem cells (CSCs), which are related to cancer malignancy (Shibue & Weinberg, 2017; Shihori Tanabe, 2015a, 2015b; Tanabe, Aoyagi, Yokozaki, & Sasaki, 2015).

EMT phenomenon is related to cancer metastasis and cancer therapy resistance (Smith & Bhowmick, 2016; Tanabe, 2013). Increase expression of enzymes that degrade the extracellular matrix components and the decrease in adhesion to the basement membrane in EMT induce the cell escape from the basement membrane and metastasis (Smith & Bhowmick, 2016). Morphological changes observed during EMT is associated with therapy resistance (Smith & Bhowmick, 2016).

## Evidence Supporting this KER

**Biological Plausibility**

The morphological and physiological changes associated with EMT are involved in invasiveness and drug resistance (Shibue & Weinberg, 2017). The EMT-activated particular carcinoma cells in primary tumors invade the surrounding stroma (Shibue & Weinberg, 2017). The EMT – activated carcinoma cells interact with the surrounding extracellular matrix protein to induce focal adhesion kinase and extracellular signal-related kinase activation, followed by the transforming growth factor beta (TGFbeta) and canonical and/or noncanonical Wnt pathways to induce cancer stem cell (CSC) properties which contribute to the drug resistance (Shibue & Weinberg, 2017).

EMT-associated down-regulation of multiple apoptotic signaling pathways induce drug efflux and slow cell proliferation to induce the general resistance of carcinoma cells to anti-cancer drugs (Shibue & Weinberg, 2017).

Snail, an EMT-related transcription factor, induces the expression of the AXL receptor tyrosine kinase, which enables the cancer cells to survive by the activation of AXL signaling triggered by the binding of its ligand growth arrest-specific protein 6 (GAS6)(Shibue & Weinberg, 2017).

The EMT-activated cells evade the lethal effect of cytotoxic T cells, which include the elevated expression of programmed cell death 1 ligand (PD-L1) which binds to the programmed cell death protein 1 (PD-1) inhibitory immune-checkpoint receptor on the cell surface of cytotoxic T cells (Shibue & Weinberg, 2017).

**Empirical Evidence**

Slug/Snai2, a *ces*-1-related zinc finger transcription factor gene, confers resistance to p53-mediated apoptosis of hematopoietic progenitors by repressing *PUMA* (also known as *BBC3*, encoding Bcl-2-binding component 3) (Inukai et al., 1999; Shibue & Weinberg, 2017; W.-S. Wu et al., 2005).

EMT activation induces the expression of multiple members of the ATP-binding cassette (ABC) transporter family, which results in the resistant to doxorubicin (Saxena, Stephens, Pathak, & Rangarajan, 2011; Shibue & Weinberg, 2017)

TGFbeta-1 induced EMT results in the acquisition of cancer stem cell (CSC) like properties (Pirozzi et al., 2011; Shibue & Weinberg, 2017).

Snail-induced EMT induces the cancer metastasis and resistance to dendritic cell-mediated immunotherapy (Kudo-Saito, Shirako, Takeuchi, & Kawakami, 2009).

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 (Chen et al., 2014).

**Uncertainties and Inconsistencies**

The reversing process of EMT, which names as mesenchymal-epithelial transition (MET), may be one of the candidates for the anti-cancer therapy, where the plasticity of the cell phenotype is of importance and under investigation (Shibue & Weinberg, 2017).

## Quantitative Understanding of the Linkage

**Response-response relationship**

Induction of EMT by TGFbeta and Twist increase the gene expression of EMT markers such as Snail, Vimentin, N-cadherin, and ABC transporters including ABCA3, ABCC1, ABCC3 and ABCC10 (Saxena et al., 2011).

Human mammary epithelial cells (HMLE) stably expressing Twist, FOXC2 or Snail demonstrates the increased the cell viability compared to control HMLE in the treatment with about 0.3, 3, 30 mM of doxorubicin, dose-dependently (Saxena et al., 2011).

**Time-scale**

The treatment with doxorubicin for 48 hours demonstrates the increase in the cell viability in Twist/FOXC2/Snail overexpressed HMLE compared to control HMLE (Saxena et al., 2011).

The inhibition of Twist or Zeb1 with small interference RNA (siRNA) induced the inhibition of the cell viability compared to control MDAMB231 cells treated with doxorubicin for 48 hours (Saxena et al., 2011).

**Known modulating factors**

ABC transporters which are related to drug resistance are overexpressed in the EMT-activated cells (Saxena et al., 2011). The expression of PD-L1, which binds to the PD-1 on the cytotoxic T cells, is up-regulated in EMT-activated cells, which results in the inhibition of cancer immunity and the resistance to cancer therapy (Shibue & Weinberg, 2017).

**Known Feedforward/Feedback loops influencing this KER**

The investigation of EMT-CSC relations is important to understand the relationship between EMT and cancer malignancy. Non-CSCs in cancer can spontaneously undergo EMT and dedifferentiate into new CSC, subsequently induce the regeneration of tumorigenic potential (Marjanovic, Weinberg, & Chaffer, 2013; Shibue & Weinberg, 2017).

The plastic CSC theory demonstrates the bidirectional conversions between non-CSCs and CSCs, which may contribute into the acquisition of cancer malignancy in EMT-activated cells (Marjanovic et al., 2013).

## References


Chen, L., Gibbons, D. L., Goswami, S., Cortez, M. A., Ahn, Y.-H., Byers, L. A., . . . Qin, F. X.-F. (2014). Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. *Nature communications, 5*, 5241-5241. doi:10.1038/ncomms6241

Du, B., & Shim, J. S. (2016). Targeting Epithelial-Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. *Molecules, 21*(7). doi:10.3390/molecules21070965

Inukai, T., Inoue, A., Kurosawa, H., Goi, K., Shinjyo, T., Ozawa, K., . . . Look, A. T. (1999). SLUG, a ces-1-Related Zinc Finger Transcription Factor Gene with Antiapoptotic Activity, Is a Downstream Target of the E2A-HLF Oncoprotein. *Molecular Cell, 4*(3), 343-352. doi:https://doi.org/10.1016/S1097-2765(00)80336-6 (https://doi.org/10.1016/S1097-2765(00)80336-6)

Kudo-Saito, C., Shirako, H., Takeuchi, T., & Kawakami, Y. (2009). Cancer Metastasis Is Accelerated through Immunosuppression during Snail-Induced EMT of Cancer Cells. *Cancer Cell, 15*(3), 195-206. doi:https://doi.org/10.1016/j.ccr.2009.01.023 (https://doi.org/10.1016/j.ccr.2009.01.023)

Marjanovic, N. D., Weinberg, R. A., & Chaffer, C. L. (2013). Cell plasticity and heterogeneity in cancer. *Clinical chemistry, 59*(1), 168-179. doi:10.1373/clinchem.2012.184655

Pirozzi, G., Tirino, V., Camerlingo, R., Franco, R., La Rocca, A., Liguori, E., . . . Rocco, G. (2011). Epithelial to mesenchymal transition by TGFβ-1 induction increases stemness characteristics in primary non small cell lung cancer cell line. *PLoS One, 6*(6), e21548-e21548. doi:10.1371/journal.pone.0021548

Saxena, M., Stephens, M. A., Pathak, H., & Rangarajan, A. (2011). Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. *Cell death & disease, 2*(7), e179-e179. doi:10.1038/cddis.2011.61

Shibue, T., & Weinberg, R. A. (2017). EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol, 14*(10), 611-629. doi:10.1038/nrclinonc.2017.44

Smith, B. N., & Bhowmick, N. A. (2016). Role of EMT in Metastasis and Therapy Resistance. *J Clin Med, 5*(2). doi:10.3390/jcm5020017

Tanabe, S. (2013). Perspectives of gene combinations in phenotype presentation. *World journal of stem cells, 5*(3), 61-67. doi:10.4252/wjsc.v5.i3.61

Tanabe, S. (2015a). Origin of cells and network information. *World journal of stem cells, 7*(3), 535-540. doi:10.4252/wjsc.v7.i3.535

Tanabe, S. (2015b). Signaling involved in stem cell reprogramming and differentiation. *World journal of stem cells, 7*(7), 992-998. doi:10.4252/wjsc.v7.i7.992

Tanabe, S., Aoyagi, K., Yokozaki, H., & Sasaki, H. (2015). Regulated genes in mesenchymal stem cells and gastric cancer. *World journal of stem cells, 7*(1), 208-222. doi:10.4252/wjsc.v7.i1.208

Wu, W.-S., Heinrichs, S., Xu, D., Garrison, S. P., Zambetti, G. P., Adams, J. M., & Look, A. T. (2005). Slug Antagonizes p53-Mediated Apoptosis of Hematopoietic Progenitors by Repressing puma. *Cell, 123*(4), 641-653. doi:https://doi.org/10.1016/j.cell.2005.09.029 (https://doi.org/10.1016/j.cell.2005.09.029)

Zhang, P., Sun, Y., & Ma, L. (2015). ZEB1: at the crossroads of epithelial-mesenchymal transition, metastasis and therapy resistance. *Cell Cycle, 14*(4), 481-487. doi:10.1080/15384101.2015.1006048