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Treatment-induced secretion of WNT16B promotes tumor growth and acquired resistance to chemotherapy

Implications for potential use of inhibitors in cancer treatment

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Innate or acquired resistance to chemotherapy presents an important and predictable challenge in cancer therapy. Malignant tumors consist of both neoplastic and benign cells such as stromal fibroblasts, which can influence the tumor's response to cytotoxic therapy. In a recent article in *Nature Medicine*, Sun et al. show that increased expression of Wnt family member wingless-type MMTV integration site family member 16B (WNT16B) by the tumor microenvironment in response to cytotoxic damage and signals through the canonical Wnt pathway to promote tumor growth and chemotherapy resistance. Such findings outline a mechanism by which cytotoxic therapies given in cyclical doses can actually augment later treatment resistance and may open the door to new areas of research and to the development of new therapeutic targets that block the DNA damage response program.

Keywords: prostate cancer, WNT16B, chemotherapy resistance, tumor microenvironment, NFκB, cytotoxic drugs, DNA damage response program

Abbreviations: WNT16B, Wnt family member wingless-type MMTV integration site family member 16B; shRNA, small hairpin RNA; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; EMT, epithelial-to-mesenchymal transition

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Resistance to chemotherapy is nearly a universal obstacle in cancer treatment and is a major contributor to treatment failure in patients with metastatic carcinomas.[1] Therapeutic resistance can occur from the initiation of treatment or as an acquired phenomenon during treatment.[2] Although most cytotoxic agents selectively target cancer cells by affecting differential tumor cell characteristics, they can still harm benign cells and disrupt normal tissue function. Thus, while extremely high doses of cytotoxic drugs would thoroughly eradicate the cancer cells, these high doses are also lethal to normal host cells. To minimize damage to noncancerous cells, both radiation and chemotherapy are administered in smaller doses and in cycles to allow normal host cells to recover in between treatments. However, this approach may not completely eliminate all of the tumor cells and may allow surviving tumor cells to develop resistance to further therapeutic attempts.[3]

Sun et al. show in a study recently published in *Nature Medicine* that DNA damage from cytotoxic therapies cause healthy stromal cells within the tumor neighborhood to secrete proteins that can influence the tumor's response to therapy.[4] The authors show that benign primary prostate fibroblasts upregulated the expression of Wnt family member wingless-type MMTV integration site family member 16B (WNT16B) by up to 64-fold as a result of the genotoxic drugs or radiation therapy. This upregulation of WNT16B in the tumor microenvironment promoted cancer therapy resistance and led to increased tumor growth and invasiveness. Previous studies have shown that WNT16B secretion normally regulates the tumor suppressor p53 in response to DNA damage and is critical for the onset of senescence.[5]

The authors confirmed that WNT16B is induced by genotoxic therapy in vivo by detecting increased prostate stromal WNT16B expression in prostatectomy tissue samples from prostate cancer patients treated with the cytotoxic drug mitoxantrone and the microtubule poison docetaxel. They also observed increased WNT16B expression in breast and ovarian fibroblasts in patients who received mitoxantrone

treatment. Of note, higher WNT16B expression in prostate stroma after exposure to mitoxantrone and docetaxel chemotherapy was associated with a much higher likelihood of cancer recurrence.

The authors then showed that increased secretion of WNT16B by DNA-damaged fibroblasts in the tumor microenvironment enhanced tumor growth and invasion. Depending on the cell line used, conditioned medium from irradiated prostate fibroblasts engineered to express WNT16B increased prostate cancer cell proliferation, migration and invasion by 15–35% compared with prostate fibroblasts expressing a WNT16B-inhibiting shRNA.

The influences of WNT16B expression in the tumor microenvironment were studied in vivo by implanting prostate cancer cells in experimental mice along with either prostate fibroblasts engineered to produce WNT16B or WNT16B-deficient prostate fibroblasts. After 8 wk, tumors growing in the presence of WNT16B-expressing fibroblasts were substantially larger than the control tumors. Furthermore, tumors averaged a 25% reduction in size when prostate cancer cells were implanted with irradiated prostate fibroblasts in which WNT16B expression was suppressed.

Having established that WNT16B promotes tumor growth through paracrine signaling, the authors demonstrated that genotoxic stress induces WNT16B expression through NFκB and signals through the canonical Wnt pathway to promote tumor growth. The upregulation of WNT16B production by prostate stromal fibroblasts was induced through NFκB binding the WNT16B gene in response to DNA damage. Of note, inhibition of NFκB signaling in DNA-damaged prostate fibroblasts reduced the effect of WNT16B-induced tumor cell proliferation. DNA-damage induced secretion of WNT16B also prompts an epithelial-to-mesenchymal transition (EMT) to increase tumor invasiveness. After exposure of prostate cancer cells to prostate fibroblasts engineered to produce WNT16B, loss of E-cadherin and gain of N-cadherin characteristic of an EMT was observed. A similar EMT was noted in breast cancer and ovarian cancer cells incubated with WNT16B-expressing prostate fibroblasts.

Finally, the authors confirmed that WNT16B promotes the resistance of prostate cancer to cytotoxic chemotherapy drugs. Coimplantation of prostate cancer cells with fibroblasts genetically engineered to produce WNT16B increased tumor size and weakened chemotherapy-induced cytotoxicity after treatment with mitoxantrone, a topoisomerase II inhibitor used clinically for the treatment of advanced prostate cancer. Conversely, inhibition of WNT16B in prostate fibroblasts reduced tumor size and heightened mitoxantrone efficacy.

Sun et al. provide insight into the role of the tumor microenvironment in acquired resistance to chemotherapy drugs. Since tumors gaining resistance to cancer treatments is a major cause of treatment failure, the mechanism of resistance is very valuable when developing ways to stop this response. Inhibiting specific stromal DNA damage secretory proteins such as WNT16B may improve responsiveness to chemotherapy. However, the practicality of WNT inhibitors is unknown due to the ubiquitous expression of WNT16B in human tissues, with highest levels in adult kidney, placenta, brain, heart and spleen.[6] Inhibiting upstream master regulators of the DNA damage response program such as NFκB may be more efficient and effective adjuncts to cytotoxic therapies, however selectivity remains a challenge when targeting NFκB, since blocking a critical component of the immune system like NFκB could promote immunosuppression. Although further studies are necessary to develop agents that block the DNA damage response program or chemotherapeutic agents that do not trigger it, the findings of Sun et al. provide considerable insight into the roots of chemotherapy resistance that may be used to guide future drug development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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