Docetaxel Resistance in Prostate Cancer: Taking It Up a Notch

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**Running Title:** Docetaxel Resistance in Prostate Cancer: Targeting Notch

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Summary

Notch signaling is implicated in prostate cancer progression and docetaxel resistance. Cui and colleagues describe the additive efficacy and mechanisms of a γ-secretase inhibitor, PF-03084014, and docetaxel in preclinical models of prostate cancer, suggesting the need for further clinical development of Notch pathway modulators in men with metastatic prostate cancer.
In this issue of *Clinical Cancer Research*, Cui and colleagues report the additive efficacy of a gamma secretase inhibitor (GSI), PF-03084014, together with docetaxel both *in vitro* and in murine models of prostate cancer, including soft tissue growth and growth and survival of tumors in bone (1). Docetaxel was the first chemotherapeutic to increase overall survival for castration resistant prostate cancer (2), but resistance to docetaxel develops commonly after about 6 months of therapy. In addition, recent data using docetaxel with androgen-deprivation therapy in the castration-sensitive metastatic setting has suggested more dramatic improvements in survival (3, 4). Several recent studies have implicated Notch signaling in docetaxel resistance, particularly in promoting epithelial plasticity, de-differentiation, and stemness properties in prostate cancer (5, 6). These studies demonstrated that targeting these stemness pathways significantly delayed prostate cancer progression, particularly with co-targeting of the Hedgehog/GLI and Notch pathways. In addition, Notch signaling has been implicated through gene expression profiling with high grade localized prostate cancer in patients (5).

Trials attempting to combine docetaxel with novel agents have been fraught with failure in over a dozen phase 3 trials, despite a range of successes in preclinical models of prostate cancer (7). While some of these failures may be attributable to challenges in decision-making in early clinical development and a lack of surrogate endpoints for early drug development decision-making, a major obstacle has also been the lack of suitable predictive preclinical models and appropriately designed experiments that recapitulate the bone microenvironment. The majority of men with lethal prostate cancer have bone metastases, while the majority of animal
models of prostate cancer do not develop bone metastases for example, limiting our ability to determine drug resistance mechanisms in this bone microenvironment. This clinical phenotype is dominant, yet animal models poorly recapitulate bone metastasis, instead relying on direct inoculation or micrometastases to bone (8, 9).

Although a century has passed since the first description of notched wings in *Drosophila melanogaster* and the Notch signaling pathway, effective modulation of this pathway in treating disease remains elusive. The Notch signaling pathway is a highly conserved signaling mechanism necessary for the development of most organs and tissues throughout many multicellular organisms (10). Mammals express 4 highly conserved Notch receptors (Notch1-4) on cell surfaces, and when bound, initiates a series of proteolytic events to release the Notch intracellular domain (NICD). The NICD then translocates into the nucleus where it binds other co-factors (CSL and MAML) to regulate transcription of Notch target genes. The 5 ligands of the Notch receptor include 3 in the Delta-like subfamily (DLL1, DLL3, and DLL4) as well as 2 in the Jagged subfamily (JAG1 and JAG2).

Dysregulation of this highly conserved, commonly expressed pathway can result in a variety of both malignant and benign disorders. While Notch receptors can act as oncogenes in some tissues, they act as tumor suppressors in others, and therefore mutations resulting in both gain of function as well as loss of function has been reported in tumorigenesis. These range from gain of function mutations in *NOTCH1* or *NOTCH2* in 5-10% of breast cancers and activating mutations in *NOTCH1* in greater than 50% of T-cell acute lymphoblastic leukemias, to loss of function NOTCH2 mutations in more than 10% of chronic myelomonocytic leukemia and loss
of function mutations in JAG1 and NOTCH2 in cholangiocarcinoma and hepatocellular carcinoma (10). In addition, Notch may act as a tumor suppressor in the skin and may be important for gastrointestinal tract maintenance, illustrating some challenges in therapeutic targeting.

The Notch receptor is tightly regulated in the cell, posing potential opportunities for pharmacologic interference (Fig. 1). Several proteolytic cleavage steps of the receptor by furin, ADAM17 secretase, and γ-secretase could be inhibited. Blocking antibodies directed at either the Notch ligands or the Notch receptors may also be useful. Intranuclear binding of MAML or CSL, the small peptide partners of NICD, could also be attempted. Inhibition of post-translation glycosylation or fucosylation processes could potentially also hinder Notch signaling. Finally, fusion peptides of Notch ligands could potentially be made to either activate or inhibit Notch signaling. Of these pharmacologic targets, the ones farthest along in clinical development include antibodies of DLL4, a Notch ligand specific for angiogenesis, as well γ-secretase inhibitors (GSIs). However, gastrointestinal, infectious, and neoplastic toxicities have limited the entrance of GSI's into larger clinical trials (10-12).

In this current issue, Cui and colleagues have shown that the GSI PF-03084014 can sensitize the growth of docetaxel-resistant cell lines (DU145R and PC3R) to docetaxel, through inhibiting Notch signaling (decreased NICD). They also made subcutaneous xenografts of the four cell lines (DU145, DU145R, PC3 and PC3R) and treated the mice with docetaxel, PF-03084014, and the combination of docetaxel and the GSI. They found that the combination had an additive effect at
inhibiting xenograft tumor growth. The investigators made bone xenografts with these cell lines, and showed that GSI monotherapy was effective at inhibiting tumor growth, while docetaxel alone was not. Through a series of elegant experiments, they showed that these tumor-inhibiting effects occurred through cellular mechanisms of decreasing cellular proliferation, blocking new blood vessel formation, and increasing apoptosis (Fig. 1). This was accompanied by modulation of parallel signaling pathways critical to prostate cancer progression, such as the PI3K/AKT pathway, BCL-2, MEK and ERK phosphorylation, and reversal of the epithelial-to-mesenchymal transition. Interestingly, Notch inhibition led to a reduction in stemness phenotypes and prostasphere formation, while docetaxel increased these properties. While still limited by the models studied and the lack of a spontaneous bone-metastatic prostate cancer model, and understanding that cell lines and mice are not men, these data suggest that further evaluation of this combination is worthy of study should a suitable drug candidate emerge for clinical testing.

There are multiple questions that deserve attention in future studies of this pathway. One is whether Notch inhibition with docetaxel reduces or prevents prostate cancer metastasis as compared to reduction in the size of existing bone metastases, and whether this combination is additive in both the castration resistant and castration sensitive settings, in order to inform on the appropriate clinical setting for study. Second is whether GSIs can be safely delivered to patients without promoting skin or gastrointestinal toxicity, while delivering pharmacodynamically active doses to tumors. Third is the mechanism(s) of
resistance to the combination of GSI and docetaxel, given recent data supporting additional stemness pathways (6).

Cui and colleagues have demonstrated that GSIs show promise as a class of therapeutics in modulating the elusive NOTCH pathway in prostate cancer, particularly in the setting of docetaxel resistance. Further preclinical and clinical development is needed, however, in order to develop GSIs as a safe and potentially efficacious therapy in this aggressive clinical setting.

References


**Figure 1.** Mechanisms of potential pharmacologic inhibition of the NOTCH receptor pathway. Proteolytic targets include the furin convertase (part 1), ADAM17 secretase (part 2), and γ-secretase (part 3). PF-03084014 is a γ-secretase inhibitor with downstream effects of increasing apoptosis, decreasing cell signaling, and decreasing epithelial-mesenchymal transitions. Other pharmacologic targets include the intranuclear binding partners of NICD, MAML (part 4) or CSL (part 5), as well as interfering with glycosylation and fucosylation (parts 6 and 7). Potential targets of blocking antibodies include DLL4 and JAGGED1 ligands (part 8) or the NOTCH receptors (part 9). Finally, peptides can be fused to the extracellular domain of NOTCH ligands to either activate or inhibit NOTCH signaling (part 10). Adapted by permission from Macmillan Publishers Ltd: *Nature Reviews Drug Discovery* (ref. 10), copyright 2014. ECD, extracellular domain; EMT, epithelial-mesenchymal transition.
**Figure 1:**

**Ligand–receptor interaction**

- Antibodies blocking cleavage

**Glycosylation, fucosylation**

- Furin convertase

**Target of PF-03084014**

**Downstream effects of PF-03084014:**
- Increase apoptosis:
  - Proapoptosis BAK and BAX
  - Prosurvival BCL-XL and BCL-2
- Decrease cell signaling:
  - Phosphorylated MEK and ERK
  - Phosphorylated PI3K
- Decrease EMT:
  - Mesenchymal markers vimentin, Snail, Slug
  - Epithelial marker E-cadherin

**주요 단어**

- JAGGED1
- DLL4
- ECD
- Fc
- NOTCH
- ADAM17 secretase
- γ-secretase
- NICD
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