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BITS AND BYTES

CCN3-EZH2-AR feedback loop: new targets for enzalutamide and castration resistant prostate cancer

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Abstract The development of castration resistant prostate cancer and anti-androgen resistance remains one of the largest hurdles in the successful treatment of prostate cancer. Therefore, the identification of dysregulated pathways contributing to this resistance and determining ways to target these mechanisms is of utmost importance. In the recent publication in *Cancer Research*, Fong et al. identify a novel role for cytoplasmic CCN3 in prostate cancer progression and enzalutamide resistance. The authors demonstrate that CCN3 expression inhibits androgen receptor signaling and thereby suppresses enzalutamide-resistant prostate cancer cell proliferation, colony formation, and xenograft tumor growth. The data from this manuscript highlight an intriguing potential therapeutic target for the treatment of CRPC and are a critical step forwards towards treating enzalutamide resistant prostate cancer.

Keywords Prostate cancer · Enzalutamide · CCN3 · EZH2 · AR

Androgen deprivation therapy (ADT) is the initial line of treatment for prostate cancer and functions by reducing circulating androgen levels to reduce tumor growth. While androgen deprivation therapy is initially effective at inhibiting prostate cancer growth, after 2–3 years of treatment, most patients will progress to castration resistant prostate cancer (CRPC) and their tumors will continue to grow (Cookson et al. 2013; Saad and Hotte 2010). CRPC is heralded by hyper-activated androgen receptor (AR) signaling leading to the transcription of downstream target genes and tumor growth despite insignificant levels of androgen present in the patient. In a recent publication of *Cancer Research,* Fong et al. identify and describe a novel regulator of androgen signaling with implications as a new target for treating CRPC and enzalutamide resistant prostate cancer by inhibiting AR signaling (Fong et al. 2016). Previously this group had determined that CCN3/NOV is one of the most strongly inhibited genes attributed to AR mediated transcriptional repression through the epigenetic silencer EZH2 (Wu et al. 2014). In the present study, Fong et al. expand upon their previous works and further elucidate the role of CCN3, specifically cytoplasmic CCN3, in CRPC and enzalutamide resistant prostate cancer.

Enzalutamide is regularly prescribed for the treatment of CRPC however one fourth of patients receiving enzalutamide fail to respond to initial treatment and even patients who initially benefit from treatment develop drug resistance within 24 months of initial exposure (Scher et al. 2012). Due to the lack of currently available treatment options, this form of the disease is often lethal and the identification of effective treatments is vital. There are a number of known mechanisms contributing to enzalutamide resistance, many of which are highly associated with aberrant and hyper-activated androgen signaling similar to the development of CRPC. Specifically, enzalutamide resistant cells have significantly increased expression of enzymes contributing to androgen synthesis such as AKR1C3. Additionally, they express mutated forms of the AR that promote gain-of-function as well as constitutively active AR splice variants which do not require ligand to become activated (Armstrong and Gao 2015). Each of these leads to an increase in AR signaling which Fong et al. suggest may be blocked by overexpression of CCN3.

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CCN3 belongs to the CCN family of matrix proteins. It has been associated with a number of cancer types and can influence a variety of cellular functions including proliferation, adhesion, migration and differentiation (Perbal 2004, 2013). Classically, this protein has been regarded as primarily a secreted protein however several studies have also observed cytoplasmic and nuclear expression (Kyurkchiev et al. 2004; Sin et al. 2009). The role of CCN3 in these fractions is poorly understood, particularly in respect to prostate cancer where CCN3 research has focused on AR-negative cancers and secreted CCN3. Fong et al. address a serious gap in the scientific understanding of CCN3 in prostate cancer by focusing their efforts on determining the effects of cytoplasmic CCN3 on AR-positive prostate cancer and androgen signaling (Fong et al. 2016).

The first question the authors ask is whether cytoplasmic CCN3 can modulate AR signaling. To address this, they developed CCN3 inducible-expression LNCaP and C4-2B cells and performed microarray analyses. Interestingly, this revealed that CCN3 expression coincided with alterations to steroid metabolism and hormone biosynthesis related genes. This suggests that CCN3 could in fact impact androgen signaling. Specifically, they found that CCN3 overexpression resulted in a reduction of androgen-stimulated gene expression and an induction of the expression of androgen-repressed genes. These data are critical because they demonstrate for the first time that in addition to AR activation suppressing CCN3 expression, CCN3 expression in turn modulates AR signaling resulting in a potential feedback loop which may perpetuate prostate cancer progression to CRPC or drug resistance. In LNCaP cells, the authors found that overexpression of cytoplasmic CCN3, but not addition of synthetic secretory CCN3 to the culture media, inhibited cell growth. To further highlight the differences between cytoplasmic and secretory CCN3, the authors also observed that overexpression of cytoplasmic CCN3, but not secretory, could reduce cell invasion as determined by Boyden chamber assay. Together, these data delineate unique actions of differentially localized CCN3 and demonstrate that increased cytoplasmic CCN3 expression may hold potential for reducing prostate tumor growth through reduction in AR signaling.

Mechanistically, Fong et al. further determined that CCN3 inhibited AR signaling by prohibiting AR nuclear translocation. This fact gained further significance when they demonstrated that translocation of not only full length AR, but also that of AR variants lacking the ligand binding domain. Overexpression of AR variants, such as ARv7, which are constitutively active in the absence of ligand, is highly associated with CRPC (Sun et al. 2010). In fact, nearly all CRPC lines display some level of AR variant expression and CWR22Rv1 cells have nearly equal expression of full length AR and AR variants. Clinically, it has been demonstrated that ARv7 expression in patients treated with enzalutamide or another anti-androgen therapy, abiraterone, correlates to lower PSA response, shorter progression-free and overall survival compared to men who do not express ARv7 (Antonarakis et al. 2014). The importance of the fact that CCN3 can prevent translocation of these AR variants to the nucleus and therefore inhibit the transcription of downstream target genes cannot be overstated. It provides a unique potential target to improve outcomes in patients with both CRPC and anti-androgen resistant disease.

To further determine the functionality of CCN3 in inhibiting AR nuclear translocation, Fong et al. next asked how CCN3 interacted with the AR to stop its translocation. In order to test their hypothesis that CCN3 and the AR physically interacted with one another, the authors conducted co-immunoprecipitation assays against CCN3 to see if AR could be detected in the precipitation complex. CCN3 was positively identified to physically interact with the AR and additional experiments determined that it is the N-terminus of the AR that is involved in the interaction. Consistent with their observations that CCN3 inhibits AR signaling, the authors also determined that CCN3 impedes binding of AR to target gene enhancer regions and an overall reduction in AR binding to the DNA using ChIP-seq and ChIP-qPCR.

Lastly, the authors used in vivo model systems to assess the functionality of CCN3 in a whole organism and found that CCN3 knockdown cells produced larger tumor xenografts than control cells. Of more interest, however, are their models using enzalutamide resistant prostate cancer cells. They specifically used this model in order to better understand the role of CCN3 in AR signaling due to the fact that enzalutamide resistant cells have increased AR signaling compared to enzalutamide sensitive cells. Both in vitro and in vivo, CCN3 overexpression was capable of inhibiting enzalutamide resistant prostate cancer cell growth. Furthermore, CCN3 overexpression was observed to reduce AR nuclear expression in vivo suggesting that its ability to inhibit AR nuclear translocation is not in vitro model specific.

Altogether, the data from this study are a critical step forward in understanding the development of not only CRPC, but enzalutamide resistance as well. Altered AR signaling is a hallmark of both in prostate cancer. The current work by Fong et al. suggests that reduced expression of CCN3, which is observed in clinical samples of CRPC compared to hormone responsive prostate cancer, could be an important piece of the androgen signaling axis that becomes dysregulated as prostate cancer progresses. They thoroughly demonstrate that re-introducing CCN3 expression in CRPC cells impedes AR nuclear translocation and signaling and can inhibit prostate cancer cell growth and reverse enzalutamide resistance. Combined with their previous work in which they demonstrated that EZH2 expression inhibits CCN3 expression, the authors uncover an intriguing potential therapeutic target for the treatment of CRPC and enzalutamide resistant prostate cancer.

References

Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, Chen Y, Mohammad TA, Chen Y, Fedor HL et al (2014) AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 371:1028–1038

Armstrong CM, Gao AC (2015) Drug resistance in castration resistant prostate cancer: resistance mechanisms and emerging treatment strategies. Am J Clin Exp Urol 3:64–76

Cookson MS, Roth BJ, Dahm P, Engstrom C, Freedland SJ, Hussain M, Lin DW, Lowrance WT, Murad MH, Oh WK et al (2013) Castration-resistant prostate cancer: AUA Guideline. J Urol 190: 429–438

Fong KW, Zhao JC, Kim J, Li S, Yang YA, Song B, Rittie L, Hu M, Yang XJ, Perbal B et al (2016) Polycomb-mediated disruption of an androgen receptor feedback loop drives castration-resistant prostate cancer. Cancer Res. doi:10.1158/0008-5472.CAN-16-1949

Kyurkchiev S, Yeger H, Bleau AM, Perbal B (2004) Potential cellular conformations of the CCN3(NOV) protein. Cell Commun Signal 2:9

Perbal B (2004) CCN proteins: multifunctional signalling regulators. Lancet 363:62–64

Perbal B (2013) CCN proteins: a centralized communication network. J Cell Commun Signal 7:169–177

Saad F, Hotte SJ (2010) Guidelines for the management of castrate-resistant prostate cancer. Can Urol Assoc J 4:380–384

Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, de Wit R, Mulders P, Chi KN, Shore ND et al (2012) Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 367:1187–1197

Sin WC, Tse M, Planque N, Perbal B, Lampe PD, Naus CC (2009) Matricellular protein CCN3 (NOV) regulates actin cytoskeleton reorganization. J Biol Chem 284:29935–29944

Sun S, Sprenger CC, Vessella RL, Haugk K, Soriano K, Mostaghel EA, Page ST, Coleman IM, Nguyen HM, Sun H et al (2010) Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. J Clin Invest 120: 2715–2730

Wu L, Runkle C, Jin HJ, Yu J, Li J, Yang X, Kuzel T, Lee C, Yu J (2014) CCN3/NOV gene expression in human prostate cancer is directly suppressed by the androgen receptor. Oncogene 33:504–513