COMMENTARY

ANO1 plays a critical role in prostatic hyperplasia

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Benign prostatic hyperplasia (BPH) is a troubling problem that affects many older men worldwide (1). This condition poses a treatment challenge because current pharmacologic therapies can have untoward side effects. A better understanding of the pathogenesis of BPH would allow for the development of novel therapies for this condition. The prostate gland functions to secrete fluids that nourish and protect sperm. One of the key mechanisms that facilitate fluid secretion is chloride transport. This, therefore, provides a rationale to study molecules that regulate chloride secretion in the context of prostate (dys)function. In PNAS, Cha et al. (2) elucidate the role of Anoctamin1 (also known as ANO1 or TMEM16A) in the development of prostatic hyperplasia.

Fluid secretion is a fundamental biologic process that occurs in all secretory epithelia. The molecular underpinning of this phenomenon was ascribed to chloride transport across the epithelium, which is controlled by calcium-activated chloride channels (CaCCs) (see review in ref. 3). The molecular identity of these channels had eluded scientists for several decades until the anoctamin family of proteins was described in 2008 (4–6). The identification of Anoctamin 1 (ANO1/ TMEM16A) as a bona fide CaCC was met with great interest. Interestingly, ANO1 is also frequently amplified and overexpressed in epithelial cancers (7–10). Although it remains unclear as to why a CaCC should be overexpressed in malignancies, several studies have investigated the role of ANO1/TMEM16A in processes such as renal cyst formation and cancer progression (11).

Despite recent advances in the field, there are still several unanswered questions. We still do not understand (*i*) the mechanism(s) by which *Ano1* is regulated and (*ii*) whether chloride flux through this channel is required for the growth-promoting phenotype that is ascribed to ANO1 overexpression. Cha et al. (2) shed light on these important issues in the context of BPH and prostate cell proliferation (Fig. 1). Through an elegant set of experiments, the authors demonstrate that ANO1 plays a crucial role in the development and progression of benign prostatic hyperplasia. It is well known that the prostate gland is sensitive to testosterone and its active form dihydrotestosterone (DHT). DHT is the active form of testosterone that is generated by 5α-reductase. Therefore, 5α-reductase inhibitors, such as finasteride, are used to treat BPH. Cha et al. (2) show that treating prostate cells with DHT in vitro leads to a significant increase in *Ano1* protein expression. Importantly, inhibition of ANO1 using either siRNA or small-molecule inhibitors abrogated the effects of DHT on cell proliferation.

They subsequently interrogated the mechanism by which DHT induces ANO1 expression. Using an ANO1 promoter assay and ChIP, they identified the presence of androgen-responsive elements (AREs) within the *Ano1* promoter. The authors then went on to show that treatment of prostate cells with DHT for 18–24 h caused an increase in CaCC currents as measured by electrophysiology. These experiments demonstrated that not only was the magnitude of the current larger in treated cells (about sixfold increase) but a greater percentage of cells were responsive to calcium stimulation after DHT treatment (increased by sixfold). These data suggest that DHT treatment leads to the production of functional ANO1 that acts as a CaCC via regulation of ANO1 transcription.

To ascertain the biological consequence of these observations, the authors used an established model of BPH. Male rats were first subjected to castration and then treated with exogenous testosterone, which led to a consistent effect of prostatic hyperplasia.

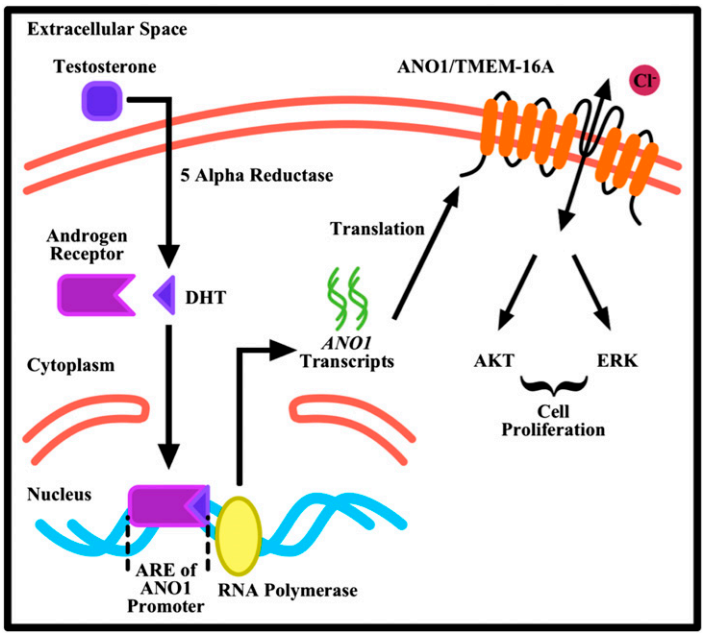

Fig. 1. Model describing the role of *Ano1* in benign prostatic hyperplasia (BPH). Testosterone is converted to the active DHT and subsequently interacts with the androgen receptor. AREs in the *Ano1* promoter lead to increased *Ano1* expression, and eventually greater expression of functional channels. There is subsequent activation of AKT and cell proliferation. The exact role of chloride ion flux through the channel still remains unclear.

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Ano1 expression was increased in the hyperplastic glands compared with vehicle-treated controls, recapitulating the in vitro observation. Similarly, the authors observed increased ANO1 expression in 51% of BPH tissue cores on a tissue array. They further went on to investigate the expression in whole tissue sections obtained from patients undergoing surgery for BPH, finding that 75% (six of eight) of tissues express ANO1. Taken together, these data bolster the argument that ANO1 plays a crucial role in BPH.

The next important question is whether ANO1 can serve as a viable therapeutic target in BPH. To address this question, the authors treated animals undergoing chronic testosterone exposure with siRNA targeting ANO1. ANO1 knockdown almost completely abrogated prostate growth induced by testosterone treatment, suggesting that testosterone-induced ANO1 expression was crucial for the development and progression of BPH. Interestingly, the effect of ANO1 knockdown on reducing prostate size was comparable to that of finasteride (a commonly used clinical treatment for BPH).

Small-molecule inhibitors against ANO1, when delivered to these animals, also reversed the hyperplastic phenotype. However, due to the lack of pharmacodynamic/kinetic data for these tool compounds, the drugs were delivered via stereotactic injection into the prostate gland. This is not yet clinically translatable. However, these data further suggest that inhibiting the chloride transport function of *Ano1* may have therapeutic benefit in ameliorating prostatic hyperplasia.

Elevated *Ano1* expression has been described in several pathologies, including cancer (10–13). Previous studies have shown that ANO1 expression leads to activation of extracellular regulated kinase1/2 (ERK1/2) and AKT (9, 10). Although existing data suggest that chloride flux through the channel may play a role in the activation of these signaling pathways, we do not know whether chloride flux is sufficient and/or necessary for the observed signaling phenotype. Furthermore, differential activation of ERK1/2 or AKT has been observed in various histopathologies, suggesting that there may be some lineage specific function of ANO1. Cha et al. (2) find that AKT, but not ERK1/2, is activated in their rat model of BPH. As expected, knockdown of ANO1 led to a significant decrease in AKT activation. When taking into account the fact that treatment with small-molecule inhibitors of ANO1 decreased gland size, we may conclude that ANO1 expression promotes prostate hyperplasia by virtue of its chloride flux. These data provide novel insights into the pathophysiology of BPH, by implicating ANO1 as a key modulator of prostatic cell growth and response to testosterone. Previous studies have investigated the role of ANO1 in cancer cell lines, and therefore in the context of genetically instability. This is the first study (to my knowledge) to investigate the role of ANO1 in benign cell proliferation, suggesting that ANO1 expression alone may be sufficient to induce cell proliferation.

The results of this study will undoubtedly stimulate future experiments to further characterize the reported findings. As the authors discuss, there are several questions raised by this study that remain unanswered. For example, how exactly does *Ano1* promote cell proliferation?

ANO1 was identified through high-resolution sequencing and in silico analysis of the 11q13 amplicon from oral cancer cells (7, 14). Since that time, gene amplification has been considered to be the major mechanism that regulates ANO1 expression. Cha et al. (2) now provide evidence that gene transcription can be regulated by extracellular signals and therefore provide new insights into the mechanism of gene regulation. Continued work is needed to elucidate the pathways involved in the regulation of ANO1 transcription, translation, and trafficking.

The effects of small-molecule inhibitors against ANO1 to prevent testosterone-induced BPH suggest that chloride flux through ANO1 is required for the observed phenotype. However, these data should be interpreted with caution. The specificity of these chloride channel inhibitors remains unclear (15). Furthermore, treatment with ANO1 inhibitors leads to a reduction in the amount of membrane-associated channels (16). Therefore, it is difficult to extrapolate data using these pharmacologic inhibitors, to conclude that chloride flux is the mechanism by which ANO1 regulates cell proliferation. In fact, it is possible that the main mechanism by which these inhibitors retard cell growth is by enhancing the degradation of protein. A detailed understanding of ANO1 biochemistry would help to delineate the mechanisms that regulate ANO1 stability and membrane insertion. Recent studies have shown that ANO1 interacts with several membrane-associated proteins including IP3R, CAMK, EGFR, and the cytoskeletal ezrin–radixin–moesin proteins (13, 17, 18). It is not out of the realm of possibility that *Ano1* exerts its progrowth effect by potentiating the signaling effect of receptor tyrosine kinases, such as EGFR.

As the authors discuss, CaCC activation can affect cell volume and/or membrane depolarization. These phenomena could in turn impact RTK signaling and provide a mechanism by which *Ano1* induced AKT activation. Further work is clearly needed to dissect these molecular mechanisms.

Cha et al. (2) clearly implicate ANO1 as a crucial player in prostate cell growth and implicate *Ano1* as a potential therapeutic target. These data may eventually be generalized to other hyperproliferative pathologies including cancer. There is now a need to develop clinically relevant small molecules that can inhibit ANO1 for potential translation into the clinical setting.

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