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INVITED RESEARCH HIGHLIGHT

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Androgen receptor splice variants and polycystic ovary syndrome: cause or effect?

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Androgen receptor (AR)-mediated androgen action provides not only a classical pivotal role in male development and functions but also a recently proven role in female reproductive physiology. Splice variants of AR are reported to occur in various androgen-sensitive cancers and now, a recent study by Wang *et al.* proposed that AR splice variants have an etiological role in polycystic ovary syndrome (PCOS). Although further investigations are required to fully appraise the significance of their discovery, these seminal findings have exciting and important implications for opening a new chapter in the understanding of the role of AR signaling in the origins and pathogenesis of PCOS.

Androgen action is classically mediated via the nuclear androgen receptor (AR), a transcription factor specified by a single X chromosome gene and which binds to androgen-response elements (ARE) in promoters of genes that direct tissue androgen effects. Recent evidence highlights that AR expression is not simply a static mediator of tissue androgen action but also displays dynamic changes flexibly adapting to pathological conditions, notably androgen deprivation.[1] A fascinating picture is emerging from research into mechanisms of ongoing androgen dependence of castration-resistant prostate cancer (CRPC). It shows that the strong androgen dependence of prostate cells is manifest not only in their functional requirement for exposure to adult male androgen levels but also in displaying an apparent androgen "addiction" whereby, when deprived of customary ambient androgen exposure, the genetic instability of prostate cancer cells fosters the evolution of multiple biochemical adaptations to maintain androgen supply by numerous alternative means.[1] Most illuminating are reports of AR splice variants, such as AR-V7, in which the ligand binding domain is deleted by RNA splicing. This leaves the remaining N-terminal moiety, still retaining the capacity to bind and activate AREs but free from restraint imposed by ligand binding to potentially become constitutively active.[2] An androgen deprived environment after castration for advanced prostate cancer enhances production of the AR-V7 variant, detectable in peripheral blood samples. This provides at least a biomarker for the intra-tumoral environment but possibly also a mechanism fuelling androgen-independent CRPC and thereby a therapeutic target.[1,2] AR splice variants have also been identified in breast and liver cancer cell lines (Hu *et al.* 2014) indicating such dynamic modification involving AR structural variants might also be involved in other androgen-sensitive human pathologies.

The most prominent effect of androgens on women's health is hyperandrogenism in women with polycystic ovary syndrome (PCOS). Worldwide, PCOS is the most frequent endocrine disorder of reproductive-aged women, causing infertility due to arrested follicular maturation and anovulation, hyperandrogenism causing acne and hirsutism, and metabolic abnormalities including obesity, insulin resistance, dyslipidemia, cardiovascular disease, and type 2 diabetes.[3,4] PCOS first became widely known after the classical publication by Stein and Leventhal 80 years ago;[5] however despite substantial research, the origins of PCOS have remained elusive. As a result, mechanism-based interventions are not feasible leaving management to rely on empirical, symptomatic treatment.

AR signaling pathways are implicated as key factors in the pathogenesis of PCOS with androgen excess being the most consistent feature.[3,4,6] The source of this androgen excess is most likely the ovary although the adrenal glands cannot be fully discounted yet. Notably, whether hyperandrogenism is cause or effect remains to be determined. The study by Wang *et al.* reported the finding of two alternative AR splice variants present in the granulosa cells of most (~62%) women with PCOS but none in non-PCOS controls.[7] They proposed that this may be an important mechanism causing ovarian hyperandrogenism and aberrant follicle development characteristic of PCOS. Thus, for the first time, AR splice variants analogous to those identified in CRPC research are proposed as a pathogenic mechanism for a nonmalignant disorder, notably as the ovarian basis for PCOS.

The authors reported two structural variants of AR both comprising in-frame modifications, one being an insertion (69bp insertion into intron 2), and the other a deletion (exon 3). Only a minority of PCOS patients display these variants, possibly reflecting their origins as somatic variants in a minority of granulosa cell clones. Interestingly, these variants were expressed more often in women with PCOS exhibiting the most severe hyperandrogenism. Furthermore, the AR variants always occur in conjunction with a larger fraction of full-length AR, a conjunction that may ameliorate the functional impact of the variants. The frequency and consistency of these variants in women with PCOS argue against random mutations and in favor of rare splice variations with an increased frequency in response to an aberrant androgenic environment. The authors reported a depth

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of molecular experimentation and modeling to show that these variants abolish or severely impair androgen action rather than enhancing it, findings that are hard to reconcile with their causing ovarian hyperandrogenism. A modest increase was observed in the DHT-induced expression of CYP17A1; however, this steroidogenic enzyme is not rate-limiting for endogenous production of the potent androgens T and DHT and was only apparent in granulosa cells transduced with the insertion variant. Rather than arguing in favor of the variants as causing the ovarian hyperandrogenism of PCOS, these findings are more consistent with them being a consequence to the hyperandrogenic state in a form of reactive, adaptation to the aberrant environment. Ultimately, however, although it is certain these AR variants must be acquired somatic changes rather than germline abnormalities, observational data alone cannot determine whether they are cause or effect of PCOS or what prompts these molecular changes.

There is strong evidence that the deletion variant may be a consequence rather than a cause of PCOS so far. The exon 3 deleted AR is a well-known mutation causing complete androgen insensitivity via germline mechanisms[8,9] as well as reported as an acquired somatic mutation in breast cancer.[10] The exon 3 deletion is also well-characterized in mice in producing complete androgen insensitivity.[11] Furthermore, in conjunction with a DHT-induced mouse model of PCOS,[12] exon 3 deletion protects against rather than causing typical PCOS features.[13] Therefore, as this is the opposite of CRPC where an androgen deprived environment enhances production of the AR-V7 splice variant, it is most likely the exon 3 deletion variant in PCOS is an adaptive response, a consequence of the aberrant hyperandrogenic follicular milieu.

The biological role of the insertional mutation is more speculative as to whether it is cause or effect. The insertion would be spliced out in the mature AR protein so, apart from changing the efficiency of AR transcription, it may not have other impacts on AR functions. The reduced functionality demonstrated conclusively by Wang *et al*. also argued in favor of this variant also being an adaptation of follicles in a PCOS ovary to an aberrant hyperandrogenic environment rather than as a cause of ovarian hyperandrogenism.

In summary, while the underlying cause of PCOS remains mysterious, this work strengthens the link between AR signaling and PCOS, and studies such as in this study, analyzing AR mechanisms remain an important approach in the search for mechanism-based treatment for PCOS. However, the claim that alternative splicing of AR in granulosa cells is the cause of the ovarian hyperandrogenism and abnormal follicle development in PCOS appears unlikely. The available data are still unable to firmly establish whether alternative AR splicing in granulosa cells is a cause or effect of PCOS. Nevertheless, just as in CRPC where prolonged androgen deprivation fosters reactive mutations in genetically unstable cancer cells, these AR variants may serve as new biomarkers of an aberrant follicular androgen environment and/or targets for novel therapies directly targeting AR signaling.

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