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RESEARCH HIGHLIGHT

The skeleton gets a (reproductive) life

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To most well-informed scientists who do not work on bone, the idea that bones determine anything other than your posture and location for the miseries of arthritis would come as quite a surprise. It would be like imagining that the walls of our home played something more than a passive role in the complexities of the lives conducted within. Yet something precisely as radical as that is what Karsenty and his colleagues boldly imagined,[1] and then provocatively established in a series of studies over the past decade.[2] Osteocalcin (OCN) is a vitamin K-dependent peptide hormone secreted by the bone-forming osteoblasts. Its carboxylated form is avidly bound to hydroxylapatite to form a major component of bone extracellular matrix. However, its uncarboxylated (uOCN) forms also enter the circulation, where it exerts systemic metabolic effects. Recent studies by Karsenty's group reveal that skeleton-derived, circulating uOCN has a significant regulatory influence on glucose and fat metabolism *via* effects on pancreatic β and fat cells,[3,4] creating an important reciprocal endocrine pathway between bone mass and energy metabolism complementing the well-known effects of catabolic states in causing bone loss. Having established a firm mechanistic basis for a role of uOCN as a contributing factor of systemic metabolism, Karsenty's group now expand that vision of bone as an active participant in the body's vital metabolism to male reproductive function.[5] While filling the ambitious panorama leads to some overinterpreted findings, the overall message of their work that the skeleton signals to the male reproductive system seems plausible. However, it is a signal rather than critical regulation, and one that primarily affects testosterone biosynthesis rather than fertility. Confusing fertility and virilisation, or treating them as interchangeable, overlooks the physiology of the testis's two interdependent functions of spermatogenesis and steroidogenesis which are nevertheless regulated, operate and malfunction quite independently. Even correcting for ambitious headlines does not diminish the extraordinary scope of this imaginative work.

Using multiple, complementary lines of *in vitro* and *in vivo* evidence, Oury *et al.*[5] show that uOCN stimulates testosterone production by Leydig cells *via* a newly identified OCN receptor, GPCR6A, expressed on Leydig cells, whereas OCN has no apparent effect on ovarian or adrenal steroidogenesis. Furthermore, global knockouts of OCN (Ocn−/−) or its proposed receptor GPCR6A[5,6] in male mice produce congruent *in vivo* features of impaired Leydig cell secretion of testosterone. Although numerous paracrine factors, many still uncharacterized, secreted by Sertoli cells, influence Leydig cell testosterone secretion,[7] other than luteinizing hormone (LH), there has been no systemic hormone previously known to directly stimulate Leydig cell testosterone secretion. Conditional loss of OCN in osteoblast cells presented strong *in vivo* evidence that bone-derived OCN induced this testicular response. In this model, transgenic Cre expression driven by the alpha1(I)-collagen promoter targeted osteoblast OCN disruption. However, transgenic Cre activity in adult testis or male reproductive tissues has not been examined in this mouse line,[8] and therefore 'leakage', notably testicular OCN disruption, is not excluded in this approach. Targeted OCN loss in Leydig cells had no effect, but involvement of other key yet sparse cells, such as the Sertoli cell (<5% of mature testis cells), was not directly examined. In addition, the mouse has three related OCN-like gene copies, previously called *OG1/OC-A*, *OG2/OC-B* and *ORG/OC-X*, within a gene cluster spanning 23 kilobases.[9] The *OG1* and *OG2* genes are predominantly expressed in bone, whereas *ORG* is highly expressed in the testes and male reproductive tract of mice.[10,11] The phenotype of Ocn−/− males reflects the loss of *OG1* and *OG2*, but not *ORG*, and possible functions of *ORG* in this new testicular pathway, or elsewhere in the reproductive tract, remain to be examined.

While it is well established that OCN influences Leydig cell testosterone secretion, the further claim that this OCN testicular action influences male fertility by local disruption of germ cell survival is questionable. The authors astutely recognized the original clue, that Ocn−/− males bred poorly may be meaningful, whereas often poor breeding in non-reproductive knockout lines is considered a nuisance to be disregarded. This study shows that such observations may provide important clues to unsuspected reproductive regulatory functions. Nevertheless, it is also possible that such findings are an epiphenomenon or may be misinterpreted. The claim of reduced male fertility of Ocn−/− male mice is based on their producing fewer and smaller litters in brief (8 weeks) breeding trials lacking quantitative survival analysis.[12–14] Yet litter frequency and size are usually strongly female-determined, according to the length of estrus cycles and numbers of oocytes ovulated. Mice with reduced spermatogenesis display normal fertility (including litter frequency and size) until spermatogenesis is reduced by >90% (i.e. <10% of normal sperm), but below that no fertilisation occurs.[15] More often, in genetic mouse models, reduced male fertility is due failure to mate when testosterone production is sufficiently impaired, especially during the critical neonatal period when male copulatory competence is established, or due to abnormal sperm function. The severe reduction in blood testosterone in the Ocn−/− male mice is consistent with impaired mating behaviour; however, the insufficient details of

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the mating trials are reported to determine whether the halved litter size (4 *vs.* 8) reflects that only half the Ocn−/− males mated and produced normal-sized litters or all mated to produced half-sized litters. Given the unimpaired sperm function and presence of >50% normal sperm numbers, the latter would be very surprising. While spermatogenesis has a critical dependence on testosterone, once spermatogenesis is initiated, it is robust and relatively insensitive to all but the most severe reductions in testosterone production.[16] Therefore, the reported subfertility of Ocn−/− male mice may be indicative of testosterone or sperm functional defects rather than the modest reduction in spermatogenesis *per se*.

Other recent work extends the complexity of potential reproductive pathways regulated by OCN–GPRC6A signalling, involving both testicular and extratesticular consideration. Pi *et al.*[17] propose a role for GPRC6A in the regulation of non-genomic androgen activity in multiple tissues, including bone and testes. Pharmacological doses of testosterone stimulated non-genomic responses, rapid extracellular signal-regulated kinase activity and Egr-1 expression, in bone marrow and testes of wild-type (WT) mice, responses that were reduced in Gprc6a−/− mice.[17] Seminal vesicles also express Gprc6a, and testosterone treatment did not restore the size of seminal vesicles in castrated Gprc6a−/− mice, unlike treated WT mice, providing evidence for direct extratesticular GPRC6A actions. Unexpectedly, testosterone administration markedly elevated circulating LH levels in Gprc6a−/− mice, in contrast to androgen-induced suppression of LH in WT males,[17] also suggesting a role for GPRC6A beyond local testis actions. Whether or not the GPRC6A-mediated and proposed non-genomic effects of pharmacological testosterone have physiological relevance, or are restricted to androgen actions has yet to be resolved. Future investigations will need to reconcile potential extratesticular actions of OCN/GPRC6A pathways, as well as changes to other steroids in such models, such as the elevated estradiol levels found in Ocn−/− males.

An outstanding question from the proposed testicular OCN receptor is how this signaling pathway interacts with the principal LH-stimulated steroidogenic pathway? While direct binding of OCN to Leydig cell GPRC6A was not demonstrated, *in vitro* and *in vivo* evidence from Karsenty's group indicates that OCN requires GPRC6A to induce cAMP signalling and elevate steroidogenesis in Leydig cells. It is intriguing that this proposed OCN pathway involves cAMP, a crucial signalling mechanism shared by the predominant LH receptor pathway. Elevated levels of circulating LH were not able to counteract the reduced steroidogenic output from Ocn−/− or Gprc6a−/− testes, suggesting that OCN and LH have distinct requirements, or that OCN effects may extend beyond Leydig cell function alone. Understanding the relationship between OCN and LH, induction of steroidogenesis remains an obvious future challenge.

Involvement of the OCN/GPRC6A pathway in male fertility provides an important additional pathway to be integrated into the already complex and integrated feedback pathways comprising the hypothalamic–pituitary–gonadal axis regulation of testicular steroidogenesis and spermatogenesis. Dissecting the role of OCN/GPRC6A-regulated pathways will need to consider the direct testicular actions of OCN or OCN-related factors and interactions with known LH receptor signalling, and the possibility of extratesticular actions by steroid-dependent regulation, or local GPRC6A-driven actions at both central and testicular levels. These new findings will no doubt stimulate new exciting research to understand the global importance of now recognized reciprocity of endocrine regulation between bone and male reproductive tissues.

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