PROGESTERONE-RESPONSIVE OSTEOPROGENITORS ARE REGULATED BY ESTROGEN AND DISTINCT FROM GLUCOCORTICOID-RESPONSIVE OSTEOPROGENITORS

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Introduction

Postmenopausal bone loss is clearly associated with a deficiency of sex steroids (1), and estrogen replacement therapy prevents it (2). Progesterone (Prog) deficiency could also be a factor, and Prog replacement therapy has indeed been shown to prevent postmenopausal bone loss and bone loss associated with ovarian dysfunction (3). In the osteoprogenitor assay system that we developed, morphologically recognizable osteoblasts and bone nodules appear in long-term rat calvaria or adult rat vertebral cell cultures at predictable and reproducible periods after plating (4-7). The uniqueness of this system is that it allows one to identify osteoprogenitors on the basis of a clearly identifiable end point, i.e. bone formation, and thus is able to identify populations of progenitors that could be heterogeneous for several other characteristics (for example, surface antigens, receptors). In our studies of these osteoprogenitors, we have discovered that, in addition to the previously found dexamethasone (Dex)-dependent and Dex-independent classes of progenitors found in calvaria of fetal rats (4), a new class of Prog-responsive progenitors was present in female-derived but not in male-derived adult cell populations (5,6). The main aim of the present study was to investigate the changes with aging of the characteristics and the sex steroid regulation of proliferation and differentiation of these osteoprogenitors and whether Prog- and Dex- responsive osteoprogenitors are two different and distinct progenitor populations.

Materials and Methods

In the present study, we used bone cell populations derived from lumbar vertebral bone of prepubertal (75g) or young adult (250g) female and male Wistar rats. Bone cell populations were isolated from vertebral body as previously described (5), plated at a plating density of $5x10^4$ cells/35mm dish and cultured in standard medium (αMEM+10% FBS and 50μg/ml ascorbic acid) with or without various concentrations of Dex, Prog, or 17β-estradiol (E2). To induce mineralization of osteoid nodules, 5mM β-glycerophosphate was added for the last 4-5 days of the culture (4). The cultures were evaluated for colonies of alkaline phosphatase (AP)-positive cells with a histochemical stain for AP and for expression of the differentiated osteoblast phenotype by von Kossa staining for mineralized bone matrix (bone nodule formation) as described previously (5). To investigate whether Prog- and Dex- responsive osteoprogenitors are two different progenitor populations, we have used replica plating (8). This technique entails the production of copies of individual colonies of cells, and thus allows study of the progeny of a single progenitor under different culture conditions. Cell populations were isolated from vertebral bone of adult female rats and plated at colony density. Colonies were duplicated by placing a polyester membrane on top of the cells on the culture dish from day 1 to 9 of culture. Duplicated colonies were cultured for an additional 29 days in medium containing either Prog or Dex and were evaluated for expression of the differentiated osteoblast phenotype by von Kossa staining for mineralized bone matrix (bone nodule formation).

Results

In cell populations derived from female rat vertebrae, Dex (10^{-10}-10^{-6}M) and Prog (10^{-8}-10^{-5}M) both induced a dose-dependent increase in the number of osteoblast colonies (bone nodules) and colonies of AP-positive cells. Bone nodule formation induced by Dex was detectable earlier than that induced by Prog, suggesting that Prog-dependent cells might be mostly early osteoprogenitors. The Prog-responsive population can not be detected in male rats after sexual maturation but is present in prepubertal rats of both sexes, and can be induced in adult male-derived populations by culturing the explants in media containing E2 (10^{-9}-10^{-8}M). The Dex-induced increase in the number of bone nodules in populations derived from prepubertal and adult rats was independent of the sex of the animal in both age categories. E2 (10^{-9}-10^{-6}M) had no significant effect on Dex-induced bone nodule formation, indicating the effect is specific for Prog-induced stimulation of bone nodule formation. When, in our replica-plating experiments, one of the duplicate colonies was cultured in Dex (10^{-8}M)-containing medium and the other in Prog (10^{-5}M)-containing medium, 5.0% of duplicates contained a bone nodule in Prog only, while 11.1% contained a bone nodule in Dex only. The percentage of duplicate colonies expressing a bone nodule in both Prog- and Dex- containing media was 3.4%. These results indicated that the majority of colonies forming bone in Prog were different from those forming bone in Dex (Chi-Square test, $X^2P=0.028$).

Discussion

The data presented indicate that in addition to the previously found Dex-dependent class of progenitors, a new class of osteoprogenitors that is responsive to Prog but not to Dex is present in female-derived bone cell populations. In addition, the results demonstrate that the Prog effects are not mediated by Prog binding to the glucocorticoid receptor and imply that in females Prog may play an important role in maintaining bone mass *in vivo* through regulating a specific class of osteoprogenitors responsive to Prog. These observations suggest the possibility that in the female skeleton one of the mechanisms to maintain bone mass is related to effects of estrogen and Prog on a class of osteoprogenitors responsive to Prog.

References

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