Viewpoint

Complementary yet distinct roles for oestrogen receptor-α and oestrogen receptor-β in mouse mammary epithelial proliferation

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Introduction

There is strong evidence that the alpha form of the oestrogen receptor (ERα) mediates the effects of oestradiol (E2) on proliferation of mammary epithelial cells although, paradoxically, it is rarely expressed in proliferating cells [1,2]. In contrast, mammary glands of mice in which the ERβ gene has been deleted develop normally. However, the role of this ER isoform in controlling growth and/or differentiation of the mammary epithelium has yet to be defined. A recent paper by Cheng and colleagues [3] examines the effects of E2, the selective oestrogen receptor modulator tamoxifen (TAM), and a novel ERβ-specific agonist, BAG, on proliferation and steroid receptor expression in the mammary glands of wild type mice and in those in which the ERβ gene has been knocked out (KO).

Steroid receptors and cell proliferation

Ovariectomised mice were used to examine the proliferative effects for 48 hours following treatment with E2, TAM or BAG. The halogenated thymidine analogue bromodeoxyuridine (BrdU) was used to label cells in S-phase 0 and 24 hours into the treatment and tissues were collected at 48 hours. This permitted the number of proliferative mammary epithelial cells that accumulated over the 48-hour treatment period to be examined. Surprisingly, all three compounds induced similar numbers of BrdU-labelled cells suggesting that TAM acts as an agonist on the mammary glands of ovariectomised mice and that BAG can elicit proliferation via ERβ. The authors concluded that both ERα and ERβ can mediate the proliferative effects of E2 on the mouse mammary epithelium.

Over the same time period, and also in mice treated continuously for 3 weeks, the effect of E2 and TAM on steroid receptor expression was examined using immunohistochemistry and Western blotting. ERα was clearly shown to be down-regulated by E2 in both wild type and ERβ KO animals, but little effect on ERβ or the E2-regulated gene, progesterone receptor (PR), expression could be demonstrated. Interestingly, TAM differed from E2 in that it had little effect on ERα expression, but reduced ERβ expression by about half. Over the 48 hours following injection of E2, the cell cycle-associated protein cyclin D1 accumulated and then disappeared from cell nuclei with similar kinetics to the loss and re-expression of ERα.

These latter data provided the rationale for a re-examination of the relationship between proliferating cells and ERα or PR expression. When BrdU-labelling was carried out shortly before removing and processing the tissue for immunohistochemistry, no association between expression of either ERα or PR and BrdU uptake could be demonstrated. However, when labelling was carried out 2 days prior to analysis, about 20% of BrdU-positive cells were PR-positive. This result suggests that these dually labelled cells are daughter cells of those that have proliferated.

Previous studies that have generated similar data conclude that proliferating cells respond to E2 indirectly via paracrine or juxtacrine signalling [1,2]. However, in this paper, the conclusion is that ERα is down-regulated in proliferating cells in response to E2. If this is the case, then an association between ERα and proliferating cells should be demonstrable in the TAM-treated samples where proliferation is increased but ERα is not down-regulated. Secondly, since ERα and PR are known to be co-expressed [1] but there is no downregulation of PR, large numbers of BrdU-positive, PR-positive cells should be detected. This is not the case.

BAG = ERβ agonist; BrdU = bromodeoxyuridine; E2 = oestradiol; ER = oestrogen receptor; KO = knock-out; PR = progesterone receptor; TAM = tamoxifen.

Conclusion

The article provides important new insights into E2-induced proliferation mediated by both ERα and -β. However, the conclusion that ERα is expressed and then down-regulated in proliferating cells is not supported by the data presented here.

The most interesting result is that proliferation of mammary epithelial cells can be increased by the novel ERβ-specific agonist BAG, suggesting that ERβ does, indeed, play a role in mediating the effects of E2. They also show for the first time *in vivo* that ERα is rapidly lost from the nucleus following E2 and that ERβ is up-regulated by E2, but down-regulated by TAM.

Other findings confirm the work of other groups and demonstrate that: ERα is epithelial whereas ERβ is both epithelial and stromal [4]; steroid receptor expression and proliferation are dissociated but daughter cells express receptors [5,6]; and ERβ is expressed by some proliferating cells [7].

Rather than indicating a direct role for ERα and PR in proliferating cells, the finding that daughter cells express these steroid receptors suggests existence of a distinct lineage that may act as a sensor cell population and mediate the proliferative effects of E2 and/or progesterone in the mammary gland.

Competing interests

None declared.

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