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Interactions between FGF2 and TGFβ-family Members in Control of the Onset of Mouse Spermatogenesis

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The effect of different TGFβ-family members, namely TGFβ1, activin, inhibin, Mullerian inhibitory substance (MIS) — holo MIS, N- and C-terminal domains, on the onset of mouse spermatogenesis in presence of FGF2 was studied. 2-day-old mouse testes were cultured 24 hours in vitro in DMEM supplemented with maximally stimulating dose of FGF2 and different TGFβ family members. DNA synthesis in quiescent mouse spermatogonia was detected by means of immunocytochemistry using Cell proliferation kit (Amersham). It was registered that TGFβ1, inhibin, holo MIS and C-terminal domain of MIS down-regulate FGF2-stimulated germ cell proliferation while activin and N-terminal domain of MIS were not effective. The obtained results show that interactions between FGF2 and TGFβ family members play a key role in controlling the onset of mouse spermatogenesis.

Key words: growth factors, mouse spermatogenesis.

Introduction

Factors regulating the proliferation of spermatogonial stem cells and thereby providing the lineage of germ cells required to generate enormous number of sperm, are the object of intensive studies lately. In adult testes the progenitor stem cells are the source for mitotically proliferating spermatogonia and in turn the differentiating spermatocytes and spermatids [1]. It is suggested that FSH together with local growth factors are involved in the renewal of male germ cell proliferation. In immature testis few days before and after birth prospermatogonial cells enter nonmitotic quiescent phase. About day 3-4 after birth in mouse testis quiescent spermatogonia undergo nearly coordinately a series of cell divisions and this event marks the onset of spermatogenesis. Previously we showed that FGF2, LIF and TGFβ family members are involved in regulation of the onset of rat spermatogenesis [3, 4]. In the present paper we present a data about the interactions between FGF2 and TGFβ family members, namely inhibin, activin, TGFβ1, MIS (holo MIS, N- and C-terminal domains) in control of the onset of mouse spermatogenesis.

Material and Methods

2-day-old mice testes were cut into 2 segments, placed on permeable celloidine membranes and cultured in organ culture dishes, containing DMEM, supplemented with 2% BSA and 5-bromo-2-deoxyuridine (BrdU) (negative control). In positive controls the medium was supplemented additionally with FGF2: maximal effective dose of 1 ng/ml. In experimental groups in addition to FGF2, the medium was supplemented with different doses inhibin, activin, TGFβ1, holo MIS, N- or C-terminal domains of MIS. The explants were cultured 24 h, immersed in OCT compound, snap-frozen and cryosectioned at 5 μm. Detection of BrdU incorporation was achieved immunocytochemically by using Cell proliferation kit (Amersham). Labelled germ cell nuclei were counted and dose-response curves were prepared.

Results

In the testis of immature mouse spermatogonial cells are situated centrally in the seminiferous cords. After culturing of 2-day-old mouse testes 24 hrs in presence of maximally stimulating dose of 1ng/ml FGF2, the percentage of proliferating spermatogonial cells increases up to 50% (Fig 1A). The effect of different doses of TGFb family members (inhibin, activin, TGFβ1, MIS) applied in combination with 1ng/ml FGF2 showed that inhibin, TGFβ1, holo MIS and C-terminal domain of MIS

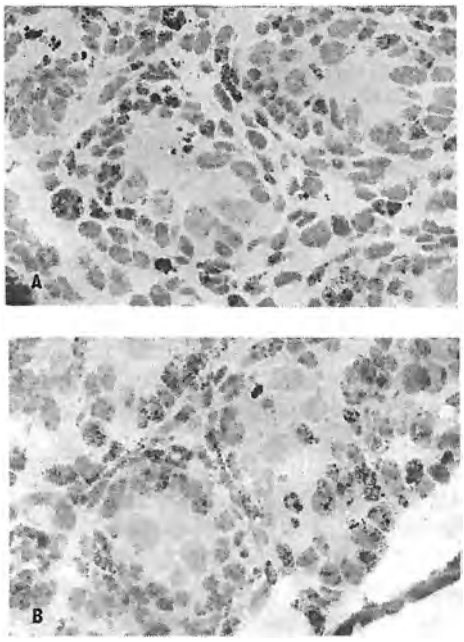

Fig.1. Light micrographs of stained frozen sections prepared from 2-day-old mouse testes after incubation 24 hrs with BrdU in presence of 1 ng/ml FGF2 (A) — positive control, or in presence of 1ng/ml FGF2 and 100ng/ml inhibin (B). × 100

down-regulate FGF2-stimulated spermatogonial proliferation. According to prepared dose-response curves (not shown), the percentage of labelled germ cells decreases up to negative controls — about 10% (Fig. 1B).

Discussion

We have shown previously that activin, together with FGF2 and LIF may positively regulate spermatogonial proliferation in mammalian testis [3]. These data support the finding about the expression of activin IIB receptors on type A spermatogonia in prepubertal testes [2]. The suppressive effect of holo MIS and C-terminal domain on FGF2-stimulated spermatogonial proliferation confirm the suggestion that C-terminal domain is the active one but not N-terminal. It is known that MIS concentration decreases during puberty and coincides with rapid germ cell proliferation stimulated by other growth factors including FGF2. In addition FGF2 stimulates Leydig cell steroidogenesis which positively regulate germ cell proliferation. In addition to MIS, an opposite relationship exists between TGFβ1 and Inhibin on one side and FGF2 from the other side. Thus interactions between TGFβ family members and FGF2 participate actively in control of the onset of mouse spermatogenesis.

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