Immunohistochemical localization of transforming growth factor-α and epidermal growth factor-receptor in the mesonephros and metanephros of the chicken

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Summary. Transforming growth factor-alpha (TGF-α) is a polypeptide related to epidermal growth factor (EGF). Both bind to EGF-receptor (EGF-R) to carry out their function in a variety of tissues and cell lines. Several studies have shown their presence in mammalian kidney, however, nothing has to date been stated concerning their existence in avian kidney. Expression of TGF-α and EGF-R is reported here for the first time during the development of the chicken kidney. Using immunohistochemical techniques, we identified a TGF-α (but not EGF) in mesonephric distal tubule cells from day 8 to day 20 of embryonic development and in metanephric distal tubule cells from day 14 of embryonic development to the adult. The histochemical characteristics of these cells and their histological localization suggest that they may be the “principal cells” of the distal tubules. Similarly, EGF-R was found in mesonephric proximal tubule cells from day 7 to day 18 of embryonic development and in metanephric proximal tubule cells from day 13 of embryonic development up to adult stages. The coexistence of both TGF-α and EGF-R from the onset of development of mesonephros and metanephros supports their possible role in mechanisms of proliferation and differentiation of the cells of these organs.

Key words: Transforming growth factor-alpha – Epidermal growth factor-receptor – Mesonephros – Metanephros – Domestic fowl

Transforming growth factor-alpha (TGF-α) described in mammals is a functional and 30% structural analog of epidermal growth factor (EGF). Both have the capability of binding EGF-receptor (EGF-R) in mammalian cells (Massagué 1990). TGF-α was originally isolated from retrovirally transformed 3T3 cells (DeLarco and Todaro 1978), as a factor that stimulates anchorage-independent growth. It has also been found to be secreted by chemically transformed cells (Lee et al. 1991; Pérez-Tomás et al. 1992) and a wide variety of tumor cells. The expression of TGF-α mRNA and protein has been described in developing mesonephric tubules of mouse (Wilcox and Derynck 1988). There is also evidence of local production of TGF-α in postnatal and adult rat kidney (Walker et al. 1991) and adult human kidney (Mydlo et al. 1989; Gomella et al. 1989). Previous studies on the effects of TGF-α in tubulogenesis (Taub et al. 1990), and induction of tubular hypertrophy (Avner and Sweeney 1990b) in vitro, suggest that TGF-α has possible roles in the regulation of cell proliferation and differentiation. Its involvement in these processes has been explained by means of paracrine or autocrine stimulation as a diffusible growth factor.

EGF-R is a transmembrane glycoprotein, which, when binding its ligand, leads to the initiation of pleiotropic responses resulting in DNA synthesis and cell proliferation. Many normal kidney cells express EGF-R, including glomeruli, collecting duct, and proximal tubule cells of rat (Safirstein et al. 1989), and also both normal and neoplastic human kidney tissue (Walker et al. 1991).

Little is known about the family of EGF and EGF-R in the chicken embryo. Mesiano et al. (1985) detected by radio-receptor assay and radiolmmunoassay, an endogenous EGF-like activity from day 10 to day 12 and day 18 to day 20 of incubation. Smith and McLachlan (1990) mapped production of growth factors with transforming activity in individual early embryos from the intermediate primitive streak stage of development. Moreover, Lax et al. (1988) deduced the primary structure of chicken EGF-R (CER), and showed it to be highly homologous to human EGF-R (HER).

Thus, to our knowledge, this is the first study localizing TGF-α and EGF-R in chicken kidney, from its embryonic development to adult stages.

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Materials and methods

Tissue collection and preparation

Kidneys from white leghorn chicken embryos were studied each day from day 3 to day 21 of incubation, as well as chicken kidneys from day 1 to day 30 after hatching and from male and female adults. Embryos or isolated kidneys were fixed for 24 h in either Bouin's fluid, Carnoy's fluid or 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.2. The samples were processed routinely for embedding in paraffin and 4 μm-thick sections were cut.

TGF-α and EGF-R immunohistochemistry

After deparaffinization and hydration, some sections were treated with 3% hydrogen peroxide, 10% methanol for 5 min, rinsed in 0.01 M PBS, pH 7.2, for 3 × 5 min. The slides were incubated at room temperature with normal rabbit serum (1:30 diluted in 0.01 M PBS, pH 7.2, 0.1% bovine serum albumin (BSA), 0.01% sodium azide (AzNa), Dakopatts Cat. No. X902), for 30 min to block non-specific protein binding, and overnight at 4°C with mouse monoclonal anti-TGF-α (1 μg/ml in PBS-BSA-AzNa, Oncogene-Science Cat. No. FG10), as a first layer. The anti-TGF-α recognizes a human and rat TGF-α epitope (residues 34-50). It does not show cross-reactivity with EGF (Sorvillo et al. 1990). The sections were then rinsed in PBS and incubated for 30 min at room temperature with rabbit IgG anti mouse IgG (1:80 in PBS-BSA-AzNa, Dakopatts Cat. No. Z259) as a second layer, washed with PBS, incubated in horseradish peroxidase and mouse anti-horseradish peroxidase complex (1:100 in PBS-BSA, Dakopatts Cat. No. P650) for 30 min as a third layer; washed with PBS and incubated for 5 min with 2.5 mg/100 ml 3,3'-diaminobenzidine tetrahydrochloride, dissolved in PBS plus 0.03% hydrogen peroxide at room temperature. These slides were rinsed in distilled water, counterstained with Harris' hematoxylin, dehydrated using graded ethanol solutions, cleared in xylene and mounted in DPX. Some sections were counterstained with the periodic acid Schiff (PAS) method to identify proximal tubules, since cells in this region present a PAS-positive brush border. Others were counterstained with alcian blue (pH 2.5) to identify the “mucus-secreting cells” of distal portion of the nephron.

After primary incubation with antibody against TGF-α, some sections were incubated for 45 min in fluorescein-conjugated rabbit IgG anti-mouse IgG, rinsed in PBS and mounted in glycerol:PBS (9:1) with p-phenylenediamine.

The same method was used to immunolocalize EGF-R, but using normal swine serum (1:30 in PBS-BSA-AzNa, Dakopatts Cat. No. X901) to block non-specific protein binding; rabbit polyclonal anti-EGF-R (1:30 in PBS-BSA-AzNa, Oncogene-Science Cat. No. PC19), which recognizes the human EGF-R fragment 1005–1016, as a first layer; swine IgG anti-rabbit IgG (1:100 in PBS-BSA-AzNa, Dakopatts Cat. No. Z196) as a second layer and horseradish peroxidase and rabbit anti-horseradish peroxidase complex (1:100 in PBS-BSA, Dakopatts Cat. No. Z113) as a third layer. Controls were: (1) primary incubation with PBS-BSA-AzNa; (2) primary incubation with either anti-TGF-α (1 μg/ml) preabsorbed with rat TGF-α (20 μg/ml, fragment 1–50, Bachem, Cat. No. H-5545) or anti-EGF-R (3 μg/ml) preabsorbed with human EGF-R (30 μg/ml, fragment 1005–1016, Sigma Cat. No. E-1886).

All sections were examined with a Leitz Diaplan microscope and photographed with a Wild MPS 45 Photoautomat system.

Results

Immunohistochemical localization of TGF-α

Immunoreactivity for the mouse monoclonal anti-TGF-α (residues 34-50) was found in cells of distal tubules (identified as PAS- and alcian blue-negative tubules) of the chicken mesonephros, from day 8 to day 20 of embryonic development (ED) (Figs. 1, 2) and also in metanephric distal tubule cells, from D14 of incubation, up to the last days of ED and even in postnatal and adult chickens (Figs. 3–6). Immunostaining was limited to a few cells in the early mesonephric (Fig. 1) and metanephric tubules. In later stages of both organs, many cells of the distal tubules were immunoreactive, showing an alternating pattern of immunostaining (Figs. 2, 4). There were positive distal tubules around glomeruli near medullary cones which belong to mammalian-type nephrons. Positive distal tubules around central veins correspond to reptilian-type nephrons (Fig. 6). The intensity of immunostaining in cells of the same tubule was similar, but different among tubules (Fig. 3). The immunostaining was almost always confined to the cytoplasm, but in tubules with stronger immunoreactivity, some nuclei appeared immunoreactive (Figs. 4, 5). Glomeruli, cells from proximal tubules (identified as PAS-positive tubules), Henle's loop and collecting ducts were never immunostained for TGF-α (Figs. 3, 6). No sex differences were observed, either in the distributional pattern or in the intensity of the immunostaining. Tissues treated with different fixatives displayed the same distribution of immunostaining. The same results were obtained by using PAP and immunofluorescence techniques. Preabsorption of anti-TGF-α with TGF-α blocked specific immunostaining (Fig. 7a, b).

Fig. 1. Day 8 of ED, mesonephros. Few TGF-α immunoreactive cells were observed dispersed throughout the epithelium in developing distal tubules. Note positive cell next to a mitotic figure (arrowhead). PAP method. ×840

Fig. 2. Day 12 of ED, mesonephros. Distal tubule cells showing an alternating pattern of immunostaining. PAP method. ×1680

Fig. 3. Day 14 of ED metanephros. Distal tubules (PAS negative) show different immunostaining intensities for TGF-α, and the proximal tubules (PAS positive) appear negative for this factor. PAP-PAS method. ×840

Fig. 4. Day 20 of ED, metanephros. TGF-α-positive cells are immunostained in the cytoplasm and nucleus. Note the strong immunoreactivity in the periphery of the nucleus (arrows). PAP-PAS method. ×1680

Fig. 5. Day 7 after hatching, metanephros. Distal tubules show a strong immunoreactivity with the antibodies against TGF-α. PAP-PAS method. ×700

Fig. 6. Adult-chicken, metanephros. Positive distal tubules of reptilian-type nephrons around a central vein (asterisk). Glomerulus (arrow) and proximal tubules (arrowheads) are negative. PAP method. ×140