Immunoreactivity of thymosin beta 4 in human foetal and adult genitourinary tract

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samples were fixed in 10% formalin, routinely processed and paraffin-embedded. Immunohistochemistry was performed on 5 μm-thick sections, using the labelled streptavidin-biotin complex system (LSAB2, Dako) in a Dako Autostainer (DakoCytomation, Carpintera, CA, USA). Heat-induced antigen retrieval was carried out by steaming unstained sections in Target Retrieval Solution (Dako TRS pH 6.1) for 30 min. Tissue sections were incubated (30 min at room temperature) with the monoclonal anti-thymosin β4 antibody (Bachem, Bubendorf, Switzerland). Sections of a reactive human adult lymph node with activated macrophages were used as positive controls. As a negative control the same procedure was applied omitting the primary antibody.

Ethics statements

The study protocol and written consent forms were approved by the Ethics Human Studies Committee of University Medical Centre of Cagliari (according to the instructions of the Declaration of Helsinki). Full written consent forms were obtained from the parents of the newborns and all rules were respected. For the specimens from adults, we obtained written consent from their next of kin.

Results

The immunostaining for Tβ4 appeared homogeneous or granular and was always restricted to the cytoplasm of positive cells. No nuclear reactivity was observed in this study. No significant differences were found in the immunohistochemical pattern for Tβ4, among the four foetuses analyzed, as well as the four adults observed in this study (Table 1). The immunoreactivity for Tβ4 in foetal kidneys paralleled the immunoreactivity in the adult. A weak diffuse cytoplasmic positivity was detected in developmental and mature ducts while the glomeruli were constantly negative (Figure 1a,b). In the bladder, a weak immunoreactivity of the peptide was observed in the immature transitional epithelium, which changed to a coarse granular reactivity in the mature epithelium of the adult bladder (Figure 2a,b). The surrounding stroma showed mild diffuse immunoreactivity of Tβ4 in the foetus and focal in adults (Figure 2b). No immunoreactivity for Tβ4 was observed in foetal endometrial glands. Only scattered stromal cells showed a weak cytoplasmic immunoreactivity (Figure 3a). A mild, granular cytoplasmic immunoreactivity of Tβ4, was present in the endometrial glands of adults, while the absence of any significant immunoreactivity was found in the stroma (Figure 3b). No reactivity for Tβ4, was detected in the foetal or adult prostatic gland epithelium (Figure 4a,b). We found a diffuse immunoreactivity of Tβ4 in the stroma of adult prostate (Figure 4b), but no reactivity was detected in the foetal prostate stroma (Figure 4a). A fine granular positivity was present in the foetal ovary (Figure 5a), while immunoreactivity of the peptide was homogeneously weak in adult oocytes (Figure 5b). Stroma was negative in the foetal or adult prostatic gland epithelium.

| Table 1. Immunoreactivity of Tβ4 in different organs and cells of the human genitourinary tract. |
|---------------------------------------------|
| **Organ**          | **Foetus** | **Adult** |
|-------------------|------------|-----------|
| Kidney Glands     | Negative   | Diffuse   |
| Kidney Stromal cells | Focal   | Diffuse   |
| Bladder Transitional epithelium | Focal | Diffuse   |
| Bladder Stroma    | Negative   | Focal     |
| Endometrium Glands | Negative  | Diffuse   |
| Endometrium Stroma cells | Focal  | Diffuse   |
| Prostate Glands   | Negative   | Focal     |
| Prostate Stroma   | Negative   | Focal     |
| Prostate Isolated cells | Focal  | Diffuse   |
| Ovary Oocyte      | Focal      | Diffuse   |
| Testicle Spermatid ducts | Negative | Focal     |
| Testicle Interstitial cells | Focal | Diffuse   |

Figure 1. Kidney. (a) Foetuses: a diffuse cytoplasmic immunoreactivity of Tβ4 is present in developing ducts (arrow). The immature glomeruli are negative (arrowhead). Scale bar: 25 μm. (b) Adults: primary and secondary ducts show a diffuse cytoplasmic immunoreactivity of Tβ4 (arrow). The glomeruli are negative (arrowhead). Scale bar: 25 μm.

Figure 2. Bladder. (a) Foetuses: the developing transitional epithelium shows a weak and diffuse cytoplasmic immunoreactivity of Tβ4 (arrow). The surrounding stroma shows a mild diffuse immunoreactivity of the peptide. Scale bar: 50 μm. (b) Adults: perinuclear coarse granules, immunoreactive for Tβ4, are detected in the cytoplasm of transitional epithelium (arrow). The stroma shows a focal positivity of the peptide (arrowhead). Scale bar: 50 μm.

Figure 3. Endometrium. (a) Foetuses: no immunoreactivity of Tβ4 is observed in developing endometrial glands (arrow). Only scattered stromal cells show a weak cytoplasmic immunoreactivity (arrowhead). Scale bar: 100 μm. Inset: scattered stromal cells with a cytoplasmic immunoreactivity (arrows). Scale bar: 25 μm. (b) Adults: a mild granular cytoplasmic immunoreactivity of Tβ4 is observed in the endometrial glands (arrow) while the surrounding stroma has no significant immunoreactivity of the peptide. Scale bar: 25 μm.
both adult and in foetal ovaries (Figure 5a,b). The interstitial cells of the adult testis were strongly positive for Tβ4, while, in the foetus, the same cells showed a mild immunoreactivity for the peptide (Figure 6a,b). Moreover, a mild reactivity of Tβ4 was observed in the spermatic ducts of the adult testicle (Figure 6b).

Discussion

This is the first comprehensive study that analyzes Tβ4 presence in the human genitourinary tract, and compares the presence of this peptide during development with its reactivity in the adult. We convincingly demonstrate that Tβ4 presence is particular for every organ and, inside each organ, it appears restricted to certain structures or to particular cell types. Many studies in experimental animals and recently in humans suggest that Tβ4 could play a relevant role during development.9,23 Our data confirms this hypothesis. In this study, we find that Tβ4 is present during intrauterine life even in the genitourinary tract, and reveal that immunoreactivity for this peptide may change during postnatal life with marked differences between foetal and adult organs. Our previous studies on Tβ4, protein presence in salivary glands9 and in the gastrointestinal tract23 first disclosed the uneven distribution of the peptide in different organs of the same system and within each organ in the different epithelial and mesenchymal structures and cells. Moreover, in recent years, Tβ4 presence has been reported to increase in different tumors,24-26 mainly in its infiltrative borders and in their metastatic stage (Nemolato et al, unpublished data). The uneven distribution of Tβ4 among different organs as well as among different cell types within each organ is confirmed by this study also in the genitourinary tract. Tβ4 was clearly present in all organs examined in this study; it was more diffuse in kidney, adult bladder and in interstitial cells of the testis.

Interestingly, we found differences in this study between foetal and mature organs. In general, we found direct associations between presence of Tβ4 and the degree of development in all the organs tested. The presence of a relationship between Tβ4 presence and the developmental status does not coincide with our previous data regarding the gastrointestinal tract.23 In previous studies, Tβ4 presence patterns were characterized by a higher presence in the foetus and by a marked decrease in the adult suggesting a major role of Tβ4 during embryogenesis. This hypothesis was confirmed by our studies of Tβ4 content in human saliva,23 which clearly showed high Tβ4 levels during gestation followed by the disappearance of the peptide in the adult saliva. Our study reinforces our hypothesis that Tβ4 functions are not totally known and stresses the different function this peptide could play in different human organs, not only during development, but also even during adult life. Some peculiar features related to the patchy Tβ4 distribution in different organs deserve to be noted in: i) kidney restriction of Tβ4 reactivity to ducts and tubules with completely spared glomeruli (Figure 1a,b); ii) a positivity in the oocytes of the ovary (Figure 5a,b); iii) immunoreactivity restricted to interstitial cells in the testis (Figure 6a,b). What is the relationship between these very different types of cells? For the time being, we have no clear answer. The immunoreactivity of oocytes of
Tβ4 in the foetal ovary and its increase in the adult oocytes indicates a role of the peptide not only in germ cell development but also in its maintenance during adult life. In this study, Tβ4 was frequently detected not only inside of cells but also in the stroma surrounding gland structures both in foetuses and in adults. Considering the fact that the extracellular forms of beta-thymosins have been shown to selectively prevent neuronal cell death,12 we can speculate that the same role could be played by Tβ4 in the genitourinary tract during development. This role should be elucidated in further studies. Finally, our study clearly shows that further work is required to better understand the presence of Tβ4, and its impacts on development and on biological behaviour of adult cells in different human organs, which confirms Tβ4, as a versatile peptide with several, mostly unknown important functions in human health and disease.

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