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Stereological analysis of age-related changes of testicular peritubular cells in men

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ABSTRACT

This work aimed to analyze quantitative changes of peritubular cells in testes of aged men. Testicular tissues were obtained from 42 aged men with advanced prostate cancer and 16 young men with biopsy, quantitatively investigated with stereological techniques with quadrate mask grid, measured the parameters volume density (V_V), numerical density on area (N_A), and numerical density (N_V) with grid test points. No significant differences were found in cell ratio, peritubular cell number per tubule, diameter of seminiferous tubules between young and old men ($p > 0.05$). Aged men had higher pathologic assignment score than that of young men, which demonstrated more severe pathologic changes ($p < 0.05$). Peritubular cell V_V and pachytene germ cell V_V increased significantly in old men compared to young men ($p < 0.05$). Sertoli cell (SC) number per tubule in two-dimensional was significantly less in aged men than that of young men, $p < 0.01$. Peritubular cell N_A, N_V decreased significantly in aged men compared to young one, $p < 0.05$. It is concluded that the stereological data of peritubular cells from three-dimensional level in testes of aged men suggest a significant decrease when compared with young men, indicating age-related changes.

1. Introduction

Human testicular peritubular cells (PCs), or peritubular myoid cells, are myofibroblast-like cells that surround the seminiferous tubules, responsible for tubular contractility and sperm transport. PCs are not only the main cellular component of the seminiferous tubule as structural cells, but also actively secrete paracrine mediators, influencing the homeostasis of the testicular environment (Maekawa et al., 1996; Albrecht, 2009). Previous studies in vitro have demonstrated that PCs secrete a number of substances including extracellular matrix components such as fibronectin, type I and IV collagens, proteoglycans (Tung et al., 1984; Skinner et al., 1985a,b) and growth factors such as transforming growth factor-α (TGFα) (Skinner et al., 1989), transforming growth factor-β (TGFβ) (Skinner and Moses, 1989), insulin-like growth factor-I (IGF-I) (Cailleau et al., 1990), activin-A (De Winter et al., 1994), and a paracrine factor of peritubular cell that mediates mesenchymal–epithelial interactions and modulates Sertoli cell functions (PmodS) (Skinner et al., 1985b; Norton and Skinner, 1989). Ciampani et al. (1992) had documented that intratesticular cell–cell interactions among the three somatic cell types of PCs, SCs and Leydig cells (LCs), provided the hormonal milieu and essential factors for germ cell development and function under the concerted actions of gonadotropins.

Age-related histological alterations in the testis include atrophy of the seminiferous tubule, reduced spermatogenic epithelium layers, thickening of the basal membrane, hernia-like protrusions of basal membrane causing dilatation of the seminiferous tubules, fibrotic thickening of the tunica albuginea, accumulation of lipofuscin in LCs and vacuolization and flattening of SCs (Richardson et al., 1995; Levitas et al., 2007). Aging has been also associated with a general loss of reproductive capacity, decreased in serum sex steroid levels, a decrease in daily sperm production, alterations in the hypothalamic–pituitary axis (Paulson et al., 2001). As one of components of the wall of seminiferous tubules, the relationship between PCs and aging remains less well defined.

Stereology is a quantitative measurement method for describing the three-dimensional (3D) structure and spatial arrangement of biologic specimens from two-dimensional (2D) thin sections (Liu et al., 2009). It has been used to evaluate parameters of seminiferous tubules, lamina propria, seminiferous epithelium, SCs and germ cells in previous studies (Bruning et al., 1993; Richardson et al., 1995; Liu et al., 2009; Silva et al., 2010). However, stereology of human testicular PCs has not been evaluated. This study proposed to analyze the quantitative data of PCs from 3D structures for age-related changes and explore the effect of male aging.

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2. Subjects and methods

2.1. Subjects

Testicular tissues were obtained by orchiectomy from 42 aged men (mean age, 74.9 ± 4.2 years) with advanced prostate cancers. The patients eligible for inclusion were: (a) not having received chemotherapy; (b) did not affect the endocrine function for pharmacological treatment. According to inclusion criteria, 16 young men (mean age: 32.6 ± 3.7 years) with biopsy for assisted reproductive detection served as controls. By pathologic observation, patients of SC only syndrome (SCOS), testicular feminization, cryptorchism were excluded. This study was approved by the ethics committee of the First Affiliated Hospital of Jinan University. All patients and subjects were informed.

Tissue samples were fixed by immersion in Bouin's fluid for about 48 h. For light microscopy, samples were subsequently processed conventionally for paraffin embedment, and 4-μm-thick sections were cut and stained with hematoxylin and eosin.

2.2. Pathologic analysis

Ten round or nearly round tubule profiles were chosen randomly and nuclei of PCs, SCs and pachytene germ cells (pGCs) were calculated at Nikon E200 light microscope (magnification 100× (1.25oil). Eight items were made to describe pathologic phenomenon, each item scores 1–5, the values are from 1 to 40 every sub-scales. Higher scores display more severe pathological changes. See details in Table 1.

2.3. Stereological measurement

Fifteen to twenty fields of view were selected randomly from each section, which uniformly acquired and snapped at 100× or 400× magnification by CCD (Micropublisher 3.3 RTV, Canada) photo software QCapture Pro 5.0, Media Cybernetics, Inc., USA). The customized image processing program was used to calculate the diameter, area and perimeter of the seminiferous tubule by IPP (Image Pro Plus 5.2, Media Cybernetics, Inc., USA). Mask grids were superimposed on images to measure parameters of testicular cells based on the stereological theory (Shen and Shen, 1997).

Dozens of tubules were needed to estimate the average diameter of the whole structure. Rectangular measurement frames were placed on each field of view to select the interested tubules. The diameter of a seminiferous tubule was defined as the shortest distance between two parallel tangent lines of the outer edge of the tubule. The mean value of the tubule diameters, which selected from a group of fields, was calculated and considered to be the estimated average diameter of seminiferous tubules of the testicular tissue (Liu et al., 2009).

Volume densities of the testicular cells were determined under image processing program, using a grid mask placed on customized images (400×). The valid intersection points (which were not located in the blank area) in the seminiferous tubules of each field were counted (P_i). The ratio of the number of valid points in the testicular cells and seminiferous tubules to the sum of all valid points was calculated to estimate V_V, N_A, N_V of testicular cells.

V_V was calculated using the following formula (Eq. (1)):

$$V_V = \frac{\sum P_{xi}}{\sum P_{ci}} \quad (1)$$

where V_V is volume density, $\sum P_{xi}$ is the total number of valid points in testicular cells counted, $\sum P_{ci}$ is the total number of valid points in the seminiferous tubules counted. Reference system is the seminiferous tubule.

N_A was calculated using the following formula (Eq. (2)):

$$N_A = \frac{\sum N_{xi}}{\sum P_{ci \cdot a^2}} \quad (2)$$

Table 1
Pathological evaluation rules of assignment score.

Items	Total tubules	Criteria	Score
1. Tubule diameter	10	<50% atrophy	1
		50–80% atrophy	3
		>80% atrophy	5
2. Cell differentiation	10	Have mature sperm	1
		Spermatid arrest	3
		Secondary spermatocyte arrest	3
		Primary spermatocyte arrest	3
		Spermatogonium arrest	5
		SC only	5
3. Cell density	10	Spermatogenic cell layers >4	1
		Spermatogenic cell layers, 2–3	3
		Spermatogenic cell layer, 0–1	5
4. Lamina propria	10	<50% fibrosis	1
		50–80% fibrosis	3
		>80% fibrosis or degeneration	5
5. Cell necrosis	10	No	0
		Few tubules little necrosis	1
		Many tubules little necrosis	2
		Many tubules much necrosis	3
6. Interstitial and vascular fibrosis	10	No	0
		Local and slight	1
		Serious and limit or Extensive and slight	2
		Serious and extensive	3
7. Interstitial cell degeneration	10	No	0
		Little and slight	1
		Serious and little or much and slight	2
		Serious and much	3
8. Interstitial edema	10	No	0
		Local and slight	1
		Serious and limit or Extensive and slight	2
		Serious and extensive	3