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P53 PROTEIN EXPRESSION IN AVIAN OVARIAN FOLLICLES

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Abstract. In the present study, we localized p53 protein in the ovary of the adult Japanese quail using immunohistochemical techniques. The best results were obtained with DO-1 monoclonal antibodies and with a heat-induced epitope retrieval method. Immunostaining was detected in cytoplasm and/or nuclei of granulosa and surface epithelial cells. In atretic follicles, p53 protein was found in a few follicular cells. Immunoreactivity was also detected in leukocytes and in the Balbiani complex of prelampbrush oocytes. It is suggested that p53 protein expression is elevated in proliferating granulosa and surface epithelial cells, and that p53 protein may be involved in granulosa cell differentiation.

Keywords: p53 protein, quail, ovary.

INTRODUCTION

P53 protein, the product of a tumor suppressor gene, is a multifunctional protein (for review see ELLEDGE & LEE, 1995). Previous studies in mammals indicate that p53 protein is an important regulator of granulosa cell fate during folliculogenesis, and that p53 protein is involved in atresia (TILLY *et al.*, 1995; AMSTERDAM *et al.*, 1996). Follicular atresia in mammals and birds is mediated via apoptosis or physiological cell death, and the majority of cells undergoing apoptosis are granulosa cells (TILLY *et al.*, 1991). A recent report on atresia in quail demonstrates that apoptosis is not the exclusive mode of active cell death (D'HERBE *et al.*, 1996). The present study focuses on the distribution of p53 protein during folliculogenesis and atresia in quail ovaries.

MATERIAL AND METHODS

Female adult Japanese quail (*Coturnix coturnix japonica*) were killed by decapitation. Tissue blocks of the ovaries and the pre- and post-ovulatory follicles were fixed in EACH fixative (PERRY-O'KEEFE *et al.*, 1990) or in 4% paraformaldehyde in 10 mM phosphate-

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buffered saline at pH 7.2. After fixation, subsamples of tissue were dissected from the walls of the pre- and post-ovulatory follicles and embedded in paraffin. Several commercially available antibodies directed against p53 protein were used (Table 1). P53 protein was localized in paraffin sections using the unlabelled antibody peroxidase-anti-peroxidase technique. Some sections were pre-treated with the pressure cooker heat-induced epitope retrieval method (NORTON *et al.*, 1994). The oocytes and follicles were classified in stages according to CALLEBAUT (1973). Paraffin sections of routinely formalin-fixed human colonic adenocarcinoma (KARAMITOPOULOU *et al.*, 1995) were used as positive controls.

TABLE 1

Antibodies directed against p53 protein

Antibody	*Dilution*	*Source*
CM1[P]	1/200	Novocastra Laboratories Ltd. (Newcastle upon Tyne, U.K.)
DO-1[M]	pd	IMMUNOTECH (Marseille, France)
	pd	DPC (Apeldoorn, The Netherlands)
	1/50	Santa Cruz Biotechnology Inc. (Santa Cruz, CA)
DO-7[M]	pd	DPC
PAb 122[M]	1/40	Boehringer Mannheim GmbH (Mannheim, Germany)
PAb 1801[M]	pd	DPC

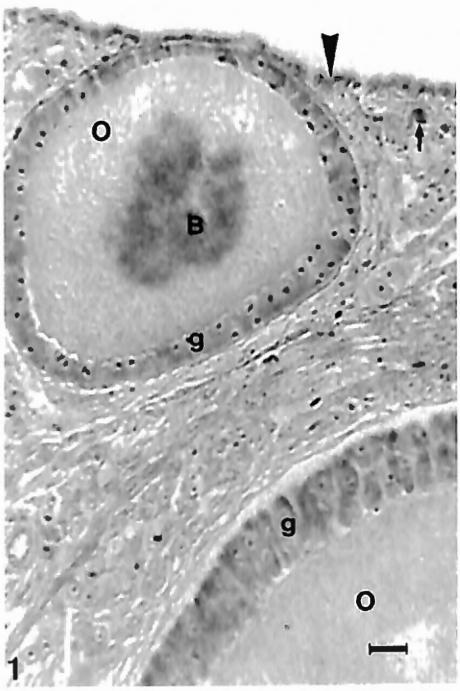
[P]: polyclonal antibody; [M]: monoclonal antibody: pd: pre-diluted.

RESULTS

The heat-induced epitope retrieval method enhanced p53 protein immunoreactivity (IR). The best results were obtained using the DO-1 monoclonal antibodies, but the CM1 and PAb 122 antibodies also gave fairly good results. No immunostaining was observed using the DO-7 and PAb 1801 antibodies. Positive staining was found with all antibodies in the adenocarcinoma sections.

IR was detected in cytoplasm and/or nuclei of granulosa cells during each stage of folliculogenesis (Figs 1-3). However, the granulosa cells of the pre-ovulatory follicles were weakly immunostained (Fig. 2). IR was also found in surface epithelial cells (Figs 1, 3), except those in the wall of pre-ovulatory follicles (Fig. 2). In atretic follicles, moderate IR was found in a few follicular cells (Fig. 4). Furthermore, p53 protein was demonstrated in the Balbiani complex of prelampbrush oocytes (Fig. 1) in a few vascular smooth muscle cells, and in leukocytes (Figs 1, 4), predominantly those present in the vicinity of atretic follicles (Fig. 4).

Figs 1-4. – Micrographs of the quail ovary, immunostained with DO-1 antiserum and counterstained with toluidine blue (bar = 10 mm). AF: atretic follicle; B: Balbiani complex of prelampbrush oocyte; g: granulosa; O: oocyte; te: theca externa; ti: theca interna; ts: tunica superficialis; arrowheads: surface epithelium; arrows: leukocytes. – 1. Prelampbrush and developing follicles: IR in granulosa and surface epithelial cells, in leukocytes, and in the Balbiani complex. – 2. Mature pre-ovulatory follicle: weak IR in the granulosa cells. – 3. Post-ovulatory follicle: IR in the granulosa cells. – 4. Atretic follicle: IR predominantly in leukocytes and in surface epithelial cells. A few follicular cells (small arrowheads) were moderately immunostained.



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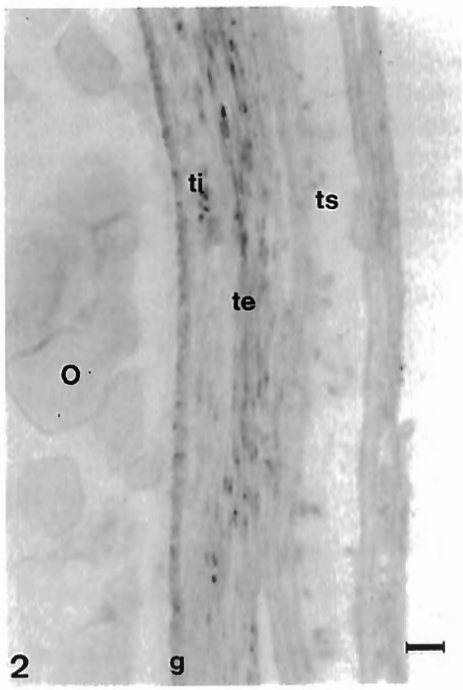

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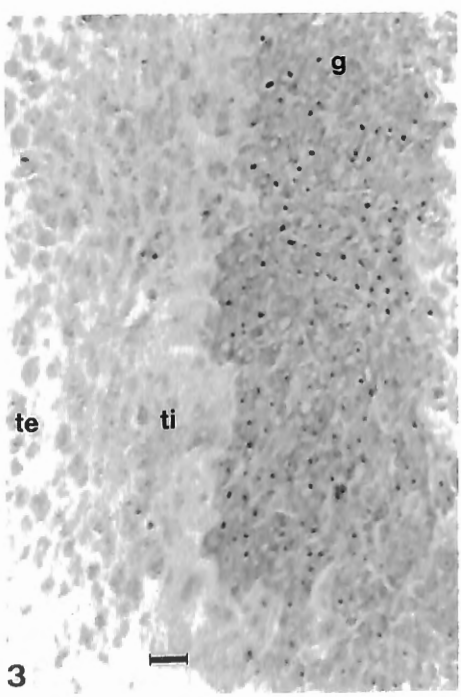

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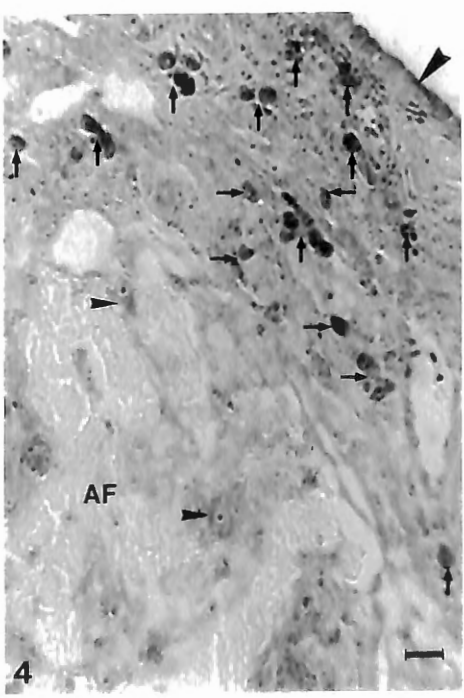

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DISCUSSION

The results with the different antibodies were probably due to the percentage homology of quail p53 protein with human p53 protein. Chicken p53 protein reveals 47% homology with human p53 protein (SOUSSI *et al.*, 1988).

In the present study, we have shown that p53 protein expression is high in proliferating granulosa and surface epithelial cells. Previous *in vitro* studies reported increased expression of p53 protein in proliferating cells (for review, see KATSUMOTO *et al.*, 1995). P53 protein initially appears in the cytoplasm of mitotic cells, then accumulates in the nucleus before the beginning of DNA synthesis, and thereafter it is no longer found in the nucleus but rather in the cytoplasm.

In the human ovary, p53 protein is detected in the nuclei of granulosa cells of preantral and antral follicles, and in the cytoplasm of surface epithelial cells (BUKOVSKY *et al.*, 1995). However, in the rat ovary, TILLY *et al.* (1995) reported that p53 protein is exclusively localized in nuclei of apoptotic granulosa cells of atretic follicles. In the quail ovary p53 protein has also been found in non-dividing and relatively stable cells: in the cytoplasm of granulosa cells of post-ovulatory and of mature pre-ovulatory follicles, and in the Balbiani complex of prelampbrush oocytes. Nuclear and nucleolar p53 protein expression is demonstrated in human oocytes of resting primary follicles and in luteinized granulosa cells (BUKOVSKY *et al.*, 1995). It is suggested that p53 protein can enhance granulosa cell differentiation and luteinization (AMSTERDAM *et al.*, 1996).

Previous findings suggest the existence of a strong correlation between apoptosis and elevated p53 protein expression in the ovary (TILLY *et al.*, 1995). AMSTERDAM *et al.* (1996) have demonstrated that p53 protein can co-operate with cAMP-generated signals in the induction of apoptosis in granulosa cells. However, we have detected a few immunostained follicular cells in atretic follicles. No clear relation between p53 protein expression and apoptosis is found.

This study supports the hypothesis (AMSTERDAM *et al.*, 1996) that p53 protein is an important regulator of proliferation of granulosa and surface epithelial cells and that p53 protein may be involved in granulosa cell differentiation.

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