

Immunohistochemical Characterization of S100A6 in the Murine Ovary

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S100 proteins comprise a large family of Ca²⁺-binding proteins and exhibit a variety of intra- and extracellular functions. Despite our growing knowledge about the biology of S100 proteins in some tissues such as brain and smooth muscle, little is known about S100 proteins in the normal mammalian reproductive tissue. In the present study, we investigated the distribution pattern of S100A6 (alternatively named calcyclin) in the murine ovary by immunohistochemical study using specific antibody. S100A6 was localized substantially in the cytoplasm of luteal cells, with concomitant expression of S100A11, another S100 protein, but not in the other type of cells such as oocytes, follicle epithelial cells (granulosa cells), and cells of stroma including theca interna cells in the murine ovary. S100A6-immunoreactive corpora lutea (CLs) were divided into two types: homogeneously and heterogeneously stained CLs, and possibly they may represent differentiating and mature CL, respectively. Our regression analysis revealed that expression level of S100A6 positively correlated with that of cytochrome P450 11A, a steroidogenic enzyme in the heterogeneously stained CL. These results suggested that S100A6 may contribute to differentiation of steroidogenic activity of luteal cells in a synergistic manner with S100A11 by facilitating some shared functions.

Key words: ovary, luteal cell, S100A6, calcyclin

I. Introduction

S100 proteins constitute a large family of small (10–14 kDa) Ca²⁺-binding proteins characterized by two EF-hands as Ca²⁺-binding sites, and exhibit a variety of functions, including cell growth and differentiation, cytoskeletal dynamics, and Ca²⁺ homeostasis (for a review see [12]). Originally, S100 α and S100 β were isolated in the bovine brain [28] and in the past decade, approximately twenty S100 genes have been identified in a variety of species, including humans and mice [23]. S100 proteins exist as homo or hetero dimers in solution, and upon Ca²⁺-binding, they undergo a large conformational change, which enables these proteins to interact with various target proteins. Through these interactions, S100 proteins transduce envi-

ronmental signals for intracellular activities such as cAMP mediated-signaling and vesicular transportation/exocytosis (for a review see [7]). In addition, some S100 proteins (*e.g.* S100B and S100A12) are secreted from cells, and bind to cell-surface receptors such as the receptor of advanced glycation endproducts (RAGE), and produce extracellular effects on neurons and inflammatory cells [3, 10, 31]. S100 proteins have also attracted much interest owing to their close association with a number of human diseases, including cancer, chronic inflammation, neurodegenerative disorders and cardiomyopathies, which suggests the potency of S100 proteins as diagnostic marker and therapeutic drug targets, although the precise mechanisms by which S100 proteins participate in disease occurrence remain largely unknown (for a review see [13]). Several lines of evidence have demonstrated S100 protein-like immunoreactivity [26, 29] and S100-gene expression by microarray analysis in the ovarian tumor [6, 14, 18]. However, little is known about the subtype-specific immunological distribution pattern of S100 proteins, particularly in the normal reproductive tissue, with the exceptions of S100A10 and S100A11 [11].

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Abbreviation: CL, corpus luteum; CYP11A, cytochrome P450 11A

Therefore, immunohistochemical analysis of S100 proteins other than S100A10 and S100A11 in the normal reproductive tissue is certainly essential for understanding the biology of S100 proteins.

S100A6 (formerly named calyculin) was first identified as a gene, the expression level of which increased when quiescent cells were stimulated to proliferate [15]. Its involvement in the process of cell cycle has been validated by several lines of evidence demonstrating reduced proliferative activities in S100A6 gene-deficient cells [4, 16, 20, 30]. S100A6 interacts with many targets, including Siah-1-interacting protein (SIP), glyceraldehydes-3-phosphatase dehydrogenase (GAPDH) and several annexins (for a review see [21]). S100A6 expression is elevated in a number of malignant tumors, such as acute myeloid leukemia, neuroblastoma and melanoma cell lines [5, 35]; therefore S100A6 may be a useful diagnostic marker for defining cancer stage. However, the precise molecular mechanism by which S100A6 regulates tumorigenesis remains unknown.

In the present study, we investigated the distribution of S100A6 in the normal murine ovary and found that S100A6 is expressed prominently in the luteal cells of the CL and that S100A6 expression positively correlated with the expression of a steroidogenic enzyme. In addition, S100A6 was also colocalized with S100A11, another S100 protein, in the luteal cells, which implies that two S100 proteins have some combined effect on the steroidogenic activity of luteal cells.

II. Materials and Methods

Animals

ICR female mice (10–12 weeks old) were obtained from the CLEA Japan (Tokyo, Japan). All mouse experiments were approved of and performed in accordance with the guidelines of the Animal Care Committee of Toho University.

Cloning and bacterial expression of mouse S100A6

Total RNA was isolated from the mouse ovary using RNA Bee (AMS Biotech., Abingdon, UK). RT-PCR was performed with ~5 µg of cDNA templates reverse-transcribed from the mouse ovary RNA. Oligonucleotide PCR primers were synthesized on the basis of the corresponding N- and C-terminal sequences of mouse S100A6 (5'-CATATGCATGCCCTCTGG-3' and 5'-CGGATCCTTATTTCAGAGCT-3' for N- and C-termini respectively). The initiation and stop codons are underlined. Amplification was performed as follows: 10 sec at 98°C, 15 sec at 61°C, and 90 sec at 68°C for 35 cycles. PCR products were subcloned into pGEM-T (Promega, Madison, WI), and found to be identical to the coding regions of S100A6 protein. The NdeI- and SpeI-digested fragment was excised and ligated with pET3a (Novagen, EMD, Darmstadt, Germany). For protein expression, the recombinant plasmid was introduced into *Escherichia coli* BL21 pLysS (Novagen). After induction of expression by IPTG, recombinant S100A6 was

purified according to the method for the purification of frog S100-like calcium binding protein described previously [27].

Western blot analysis of S100 proteins

Known amounts (50, 150 and 300 pmoles) of recombinant S100 proteins (S100A6 and S100A11) were electrophoresed in 15–20% precast gradient gel (WAKO, Osaka, Japan) and transferred onto a PVDF membrane (Immobilon P; Millipore, Bedford, MA). After blocking, blots were probed with the primary antibodies (sheep polyclonal anti-S100A6 antibody, 1:100, R&D systems, Mineapolis, MN; sheep polyclonal anti-S100A11 antibody, 1:100, Randox Life Science, Crumlin, UK). After washing, blots were reacted with HRP-conjugated secondary antibody (MP Biomedicals, Aurora, OH), and immunoreactive proteins were acquired using LAS-1000 (Fuji Film, Tokyo, Japan).

Immunohistochemistry

Ovaries were dissected from mice with normal estrous cycle, and fixed in 10% formalin, dehydrated with alcohol, embedded in paraffin, and cut at ~5 µm thickness. After blocking, the specimens were subjected to a standard immunohistochemical procedure using commercially available antibodies (sheep or rat polyclonal anti-S100A6 antibody, 1:100; sheep polyclonal anti-S100A11 antibody, 1:100; rabbit polyclonal anti-cytochrome P450 11A (CYP11A) antibody, 1:100, ProteinTech, Chicago, IL). After rinse, the specimens were treated with secondary antibodies (Alexa Fluor 594 and Alexa Fluor 488; 1:1000, Molecular Probes, Invitrogen, Carlsbad, CA) and nuclei-stained by DAPI (1 µg/ml; Dojindo, Kumamoto, Japan). Negative controls were performed by substituting the primary antibody with a non-immune normal IgG from the same sources at the same concentration. Stained specimens were observed using either a conventional fluorescence microscope (Olympus, Tokyo, Japan) or an inverted confocal laser microscope (LSM510; Carl Zeiss, Oberkochen, Germany) with an appropriate set of excitation and emission filters.

Quantitative evaluation

For quantitative analyses with a confocal microscope, the aperture, detector gain, and offset were kept constant during a series of observations, and signal intensity was set within a linear range (8 bit scale, 0–255). Images were captured at the optical slice of <0.6 µm, and quantified areas (10 µm² each, 180 pixels) were randomly chosen. To quantitatively evaluate the colocalization of S100A6 with CYP11A, the mean intensity of the cytoplasm of luteal cells within the defined area (10 µm² each) was quantified by using a software attached to the confocal laser microscope, and subtracted by the mean intensity in follicle cell as a background control, followed by the regression analysis as described elsewhere [1, 24]. Statistical significance was tested by analysis of variance (ANOVA). All quantifications were performed with lab personnel blinded to the conditions.

III. Results and Discussion

Specificity of antibodies

In general, amino acid-sequence homologies of mouse S100 proteins are not necessarily high, and occur within a range of 10–56% homology. In particular, sequence homology between S100A6 and S100A11 is substantially low (~20%). Since questions may be raised as to the antibody-specificity among subtypes of one large protein family, we cloned mouse S100A6 from the murine ovary cDNA and examined the specificity of antibodies by Western analysis using recombinant S100A6 and recombinant S100A11, another S100 protein that was subsequently focused on in this study. Anti-S100A6 antibody exhibited immunoreactivity against recombinant S100A6 but showed no cross-reaction even against excessive amount of S100A11 (6-fold quantity of S100A6) (Fig. 1A). Meanwhile, anti-S100A11 antibody reacted with recombinant S100A11, but not with the excessive amounts of S100A6 (Fig. 1B). These results essentially confirmed the specificity of the two antibodies.

Localization of S100A6 in the murine ovary

We examined the distribution pattern of S100A6 in the murine ovary by immunohistochemistry using the specific antibody described above. The result showed that S100A6 is localized substantially in the *corpus luteum* (CL in Fig. 2A, Left) but not in the follicle (F in Fig. 2A). Interestingly, S100A6+ CLs showed a varied staining appearance: all luteal cells were immunopositive in some CLs (Fig. 2A, CL demarcated by a broken line), whereas a limited number of luteal cells was immunopositive in other CLs (Fig. 2A, CL demarcated by a solid line). Furthermore, although S100A6 was present in the cytoplasm of luteal cells in both types of CLs (Fig. 2B), the intensity of S100A6-staining displayed considerable variation between the two types of CLs. The staining intensity was comparatively homogeneous in the former type of CL (referred to as homo CL), whereas it was heterogeneous in the latter type of CLs (referred to as hetero CL) (see Fig. 2B, Left; intense signal (arrow) and weak one (arrowhead)). This heterogeneous staining pattern implies that the S100A6 expression level may represent meaningful differences in the maturation process of luteal cells. Incidentally, in the hetero CL, we observed much autofluorescence (asterisk; Fig. 2B, Left), possibly due to residual red blood cells, which suggested that the hetero CL was

well vasculated and therefore represented differentiating CL at the early luteal stage [2]. Meanwhile, the homo CLs contained sinusoidal-like structure (arrowheads; Fig. 2B, Right), suggesting that these CLs were matured CLs at the mid luteal stage [2]. It should be noted that the existence of two CLs at different developmental stages in the identical ovary is due to the short estrous cycle in mice, which is consistent with another report [34].

Colocalization of S100A6 with a steroidogenic enzyme in the luteal cells

Several steroidogenic enzymes have been reported to alter their amounts during the developmental stages of the mammalian CL. For example, cytochrome P450 11A (CYP11A), a first and rate-limiting enzyme that cleaves the side chain of cholesterol producing pregnenolon, increases in the number of immunopositive cells as the CL differentiates [33]. To characterize the nature of S100A6+ luteal cells, we examined the colocalization of S100A6 with CYP11A. The result showed that S100A6 colocalized with CYP11A both in the hetero (Fig. 3A) and homo CLs (not shown). To investigate the potential correlation between expression levels of S100A6 and CYP11A, we quantified the mean intensity of S100A6- and CYP11A-immunopositive cytosolic regions within a small defined area (10 μm^2) in the hetero CLs, and performed regression analysis. The result clearly demonstrated a positive correlation between the expression levels of these two proteins (Fig. 3B; $n=100$, three mice, $F(1,32)=30$, $p<0.01$, ANOVA): S100A6 expression level was increased in parallel with CYP11A expression level, which suggested that S100A6 expression may be involved in an increase in the steroidogenic activity of luteal cells. CYP gene expression has been shown to be regulated by ubiquitous transcription factors such as AP-1 and SP-1, but more efficiently by ovary-specific transcription factors such as steroidogenic factor-1 (SF-1) and LRH-1 [8, 22]. Our computational analysis to estimate the transcription binding site in the S100A6 gene surely found both the AP-1 and SP-1 sites but unfortunately failed to detect the sites for SF-1 or LRH-1 (not shown), suggesting that the transcriptional regulation of S100A6 gene may be different from that of CYP11A.

Colocalization of S100A6 with S100A11 in luteal cells

Since we recently discovered that S100A11, another

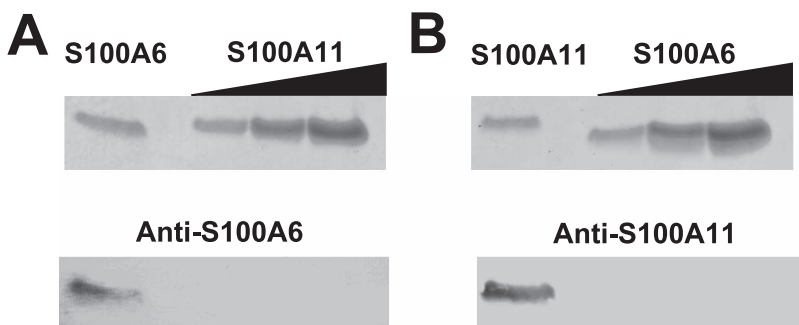


Fig. 1. Specificity of antibodies. (A) Specificity of anti-S100A6 antibody. Top: Coomassie Brilliant Blue (CBB)-stained pattern of recombinant S100A6 (50 pmoles), and recombinant S100A11 (50, 150 and 300 pmoles), Bottom: treated with anti-S100A6 antibody. (B) Specificity of anti-S100A11 antibody. Top: CBB-stained pattern of recombinant S100A11 (50 pmoles), and recombinant S100A6 (50, 150 and 300 pmoles), Bottom: treated with anti-S100A11 antibody.

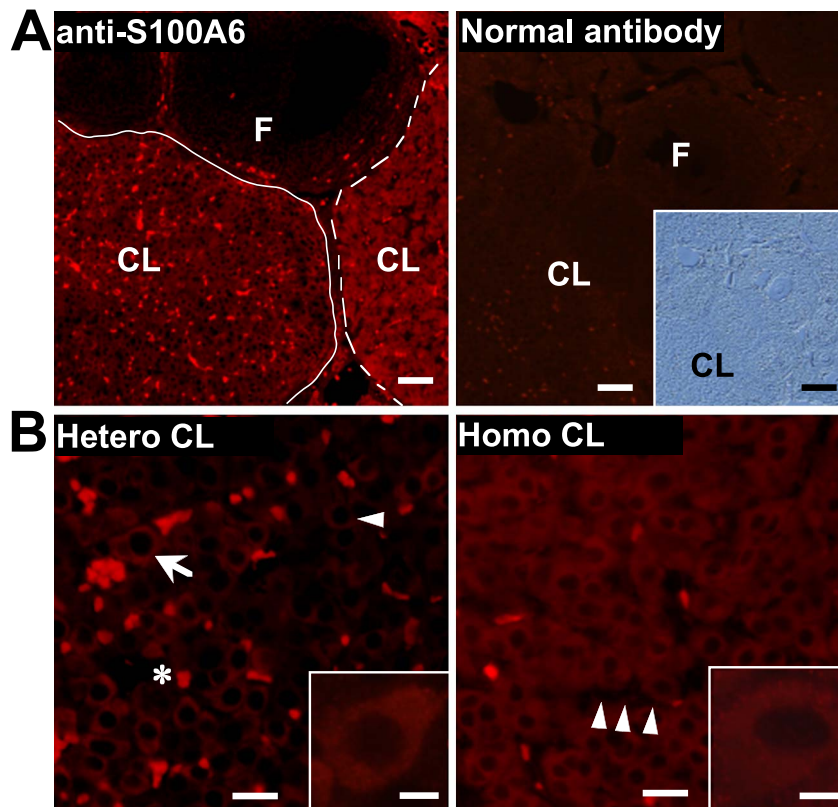


Fig. 2. Localization of S100A6 in the murine ovary. (A) Representative images of the murine ovary treated with anti-S100A6 antibody (Left) or normal sheep IgG as negative control (Right). Anti-S100A6, S100A6-immunoreactivities are prominently seen in the *corpora lutea* (CLs), but not in the follicle (F). Two types of staining pattern of CLs; homogeneously stained CL (broken line) and heterogeneously stained CL (solid line) are indicated. Bar=100 μ m. Normal antibody, no specific immunoreactivity was detected. Inset, bright field image of the ovary. Bar=100 μ m. (B) Higher-magnification images of S100A6-immunopositive luteal cells. Hetero CL, representative image of heterogeneously stained CL. S100A6 is localized prominently in the cytosol of corpus luteal cells, and immunoreactive signals are intense in some S100A6+ luteal cells (arrow) and weak in other cell (arrowhead). Asterisk denotes autofluorescence signal. Bar=20 μ m. Homo CL: representative image of homogeneously stained CL. Immunoreactive signals are comparatively homogeneous. Bar=20 μ m. Insets, representative images of S100A6-immunopositive luteal cells in homo and hetero CLs, respectively. Bar=5 μ m.

S100 protein, was localized in the cytoplasm in the luteal cells [11] similarly as seen for S100A6 in our current study, we next examined colocalization of these two S100 proteins. The result revealed that S100A6 and S100A11 colocalized in luteal cells in the hetero CL (Fig. 4), which indicated that these two proteins are concomitantly expressed in the luteal cells during luteal cell development. CL is formed via diverse cellular events involving the attenuation of cell proliferation and subsequent cell differentiation. The cyclin-dependent kinase (Cdk) inhibitor p21, the expression level of which is inhibited by β -catenin signaling, has been shown to cause cell cycle arrest, thereby initiating differentiation of luteal cells [17, 19]. S100A6 has been shown to interact with SIP, a Siah-1-interacting protein, which facilitates β -catenin degradation via ubiquitinylation in HEK293 cells, leading to p21 liberation [9, 25]. Interestingly, S100A11 has been shown to be phosphorylated in response to either exposure of high extracellular Ca^{2+} or TGF β , which leads to p21 expression, induces growth inhibition of human keratinocytes. Thus, S100A6 and S100A11 may interact with indi-

vidual targets, activating separate signaling pathways, and ultimately have a synergistic effect on the differentiation of luteal cells by enhancing p21 function. Furthermore, it is worth noting that the differentiation of luteal cells initially occurs at the stage of granulosa cells in the preovulatory follicle, following termination of the cell division by the LH surge (for a review see [32]). Our immunohistochemical studies thus far have revealed no significant immunoreactivity against S100A6 (Fig. 2A) or S100A11 [11] in granulosa cells in the preovulatory follicle, suggesting that S100A6 and S100A11 may be involved with the differentiation of luteal cells, particularly after ovulation in the CL.

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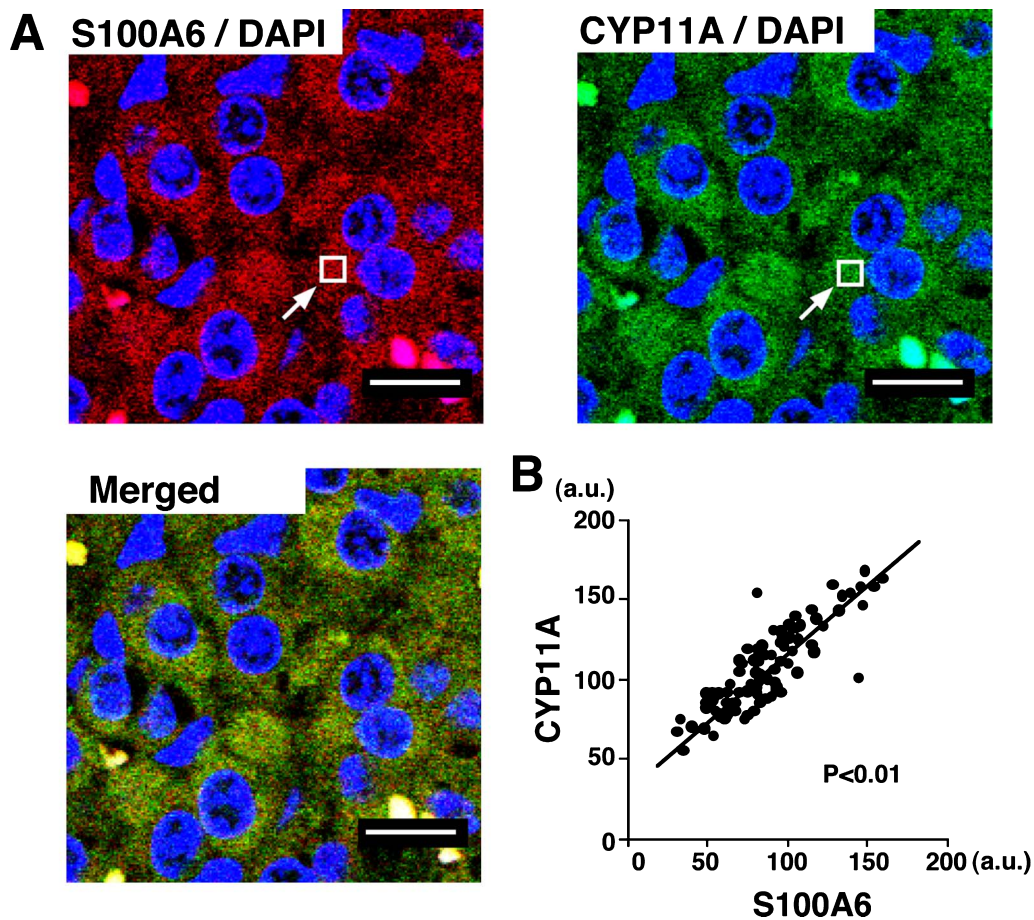


Fig. 3. Colocalization of S100A6 with cytochrome P450 11A in the luteal cells. (A) Representative confocal images of the luteal cells treated with anti-S100A6 and anti-cytochrome P450 11A (CYP11A) antibodies. S100A6/DAPI, confocal image of S100A6+ luteal cells (red). Nuclei were stained with DAPI (blue). CYP11A/DAPI, confocal image of CYP11A+ luteal cell (green) in the identical section. Merged, merged image. Bar=20 μm . (B) Regression analysis of S100A6 and CYP11A immunoreactive intensities. Immunoreactive intensities in the cytosol of luteal cells were quantified (squares in (A), 10 μm^2 per square, arrows) and subtracted by the mean intensity in the follicle. Resultant values were analyzed (n=100; three mice; a.u., optical arbitrary units). Expression levels of two proteins correlates positively ($F(1,32)=30$, $p < 0.01$, ANOVA).

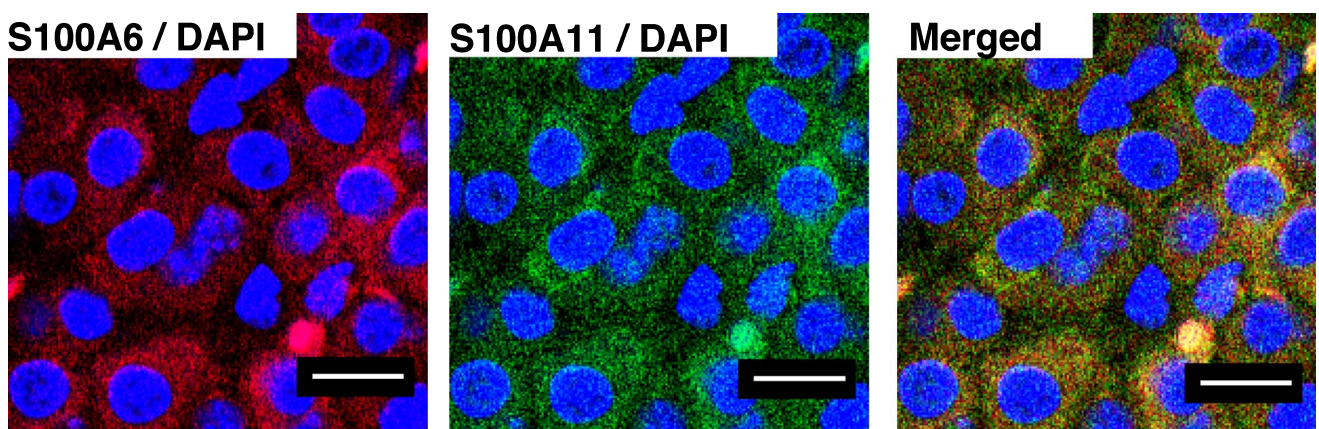


Fig. 4. Colocalization of S100A6 with S100A11 in the luteal cells. Representative confocal images of the luteal cells treated with anti-S100A6, and anti-S100A11 antibodies. S100A6/DAPI, confocal image of S100A6+ luteal cells (red). Nuclei were stained with DAPI (blue). S100A11/DAPI, confocal image of S100A11+ luteal cell (green) in the identical section. Merged, merged image. S100A6 and S100A11 colocalized with each other. Bar=20 μm .

V. References

- Antonini, J. M., Charron, T. G., Roberts, J. R., Lai, J., Blake, T. L. and Rogers, R. A. (1999) Application of laser scanning confocal microscopy in the analysis of particle-induced pulmonary fibrosis. *Toxicol. Sci.* 51; 126–134.
- Bassett, D. L. (1943) The changes in the vascular pattern of the ovary of the albino rat during the estrus cycle. *Am. J. Anat.* 73; 251–291.
- Boussac, M. and Garin, J. (2000) Calcium-dependent secretion in human neutrophils: a proteomic approach. *Electrophoresis* 21; 665–672.
- Breen, E. C. and Tang, K. (2003) Calcyclin (S100A6) regulates pulmonary fibroblast proliferation, morphology, and cytoskeletal organization in vitro. *J. Cell. Biochem.* 88; 848–854.
- Calabretta, B., Battini, R., Kaczmarek, L., de Riel, J. K. and Baserga, R. (1986) Molecular cloning of the cDNA for a growth factor-inducible gene with strong homology to S-100, a calcium-binding protein. *J. Biol. Chem.* 261; 12628–12632.
- Cross, S. S., Hamdy, F. C., Deloulme, J. C. and Rehman, I. (2005) Expression of S100 proteins in normal human tissues and common cancers using tissue microarrays: S100A6, S100A8, S100A9 and S100A11 are all overexpressed in common cancers. *Histopathology* 46; 256–269.
- Donato, R. (2003) Intracellular and extracellular roles of S100 proteins. *Microsc. Res. Tech.* 60; 540–551.
- Falender, A. E., Lanz, R., Malenfant, D., Belanger, L. and Richards, J. S. (2003) Differential expression of steroidogenic factor-1 and FTF/LRH-1 in the rodent ovary. *Endocrinology* 144; 3598–3610.
- Filipek, A. and Kuźnicki, J. (1998) Molecular cloning and expression of a mouse brain cDNA encoding a novel protein target of calcyclin. *J. Neurochem.* 70; 1793–1798.
- Gerlach, R., Demel, G., König, H. G., Gross, U., Prehn, J. H., Raabe, A., Seifert, V. and Kögel, D. (2006) Active secretion of S100B from astrocytes during metabolic stress. *Neuroscience* 141; 1697–1701.
- Hanaue, M., Miwa, N., Uebi, T., Fukuda, Y., Katagiri, Y. and Takamatsu, K. (2011) Characterization of S100A11, a suppressive factor of fertilization, in the mouse female reproductive tract. *Mol. Reprod. Dev.* 78; 91–103.
- Heizmann, C. W., Fritz, G. and Schafer, B. W. (2002) S100 proteins: structure, functions and pathology. *Front. Biosci.* 7; 1356–1368.
- Heizmann, C. W., Ackermann, G. E. and Galichet, A. (2007) Pathologies involving the S100 proteins and RAGE, In “Calcium Signalling and Disease”, ed. by E. Carafoli and M. Brini, Springer, pp. 93–138.
- Hibbs, L., Skubitz, K. M., Pambuccian, S. E., Casey, R. C., Burleson, K. M., Oegema, T. R. Jr., Thiele, J. J., Grindle, S. M., Bliss, R. L. and Skubitz, A. P. (2004) Differential gene expression in ovarian carcinoma: identification of potential biomarkers. *Am. J. Pathol.* 165; 397–414.
- Hirschhorn, R. R., Aller, P., Yuan, Z. A., Gibsin, C. W. and Baserga, R. (1984) Cell-cycle-specific cDNAs from mammalian cells temperature sensitive for growth. *Proc. Natl. Acad. Sci. USA* 81; 6004–6008.
- Hwang, R., Lee, E. J., Kim, M. H., Li, S. Z., Jin, Y. J., Rhee, Y., Kim, Y. M. and Lim, S. K. (2004) Calcyclin, a Ca²⁺ ion-binding protein, contributes to the anabolic effects of simvastatin on bone. *J. Biol. Chem.* 279; 21239–21247.
- Jirawatnotai, S., Moons, D. S., Stocco, C. O., Franks, R., Hales, D. B., Gibori, G. and Kiyokawa, H. (2003) The cyclin-dependent kinase inhibitors p27^{Kip1} and p21^{Cip1} cooperate to restrict proliferative life span in differentiating ovarian cells. *J. Biol. Chem.* 278; 17021–17027.
- Jo, M., Gieske, M. C., Payne, C. E., Wheeler-Price, S. E., Gieske, J. B., Ignatius, I. V., Curry, T. E. Jr. and Ko, C. (2004) Development and application of a rat ovarian gene expression database. *Endocrinology* 145; 5384–5396.
- Kamei, J., Toyofuku, T. and Hori, M. (2003) Negative regulation of p21 by β -catenin/TCF signaling: a novel mechanism by which cell adhesion molecules regulate cell proliferation. *Biochem. Biophys. Res. Commun.* 312; 380–387.
- Lesniak, D., Xu, Y., Deschenes, J., Lai, R., Thoms, J., Murray, D., Gosh, S., Mackey, J. R., Sabri, S. and Abdulkarim, B. (2009) Beta1-integrin circumvents the antiproliferative effects of trastuzumab in human epidermal growth factor receptor-2-positive breast cancer. *Cancer Res.* 69; 8620–8628.
- Lesniak, W., Slomnicki, L. P. and Filipek, A. (2009) S100A6—new facts and features. *Biochem. Biophys. Res. Commun.* 390; 1087–1092.
- Liu, Z. and Simpson, E. R. (1997) Steroidogenic factor 1 (SF-1) and SP1 are required for regulation of bovine CYP11A gene expression in bovine luteal cells and adrenal Y1 cells. *Mol. Endocrinol.* 11; 127–137.
- Marenholz, I., Heizmann, C. W. and Fritz, G. (2004) S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature). *Biochem. Biophys. Res. Commun.* 322; 1111–1122.
- Matsumine, M., Shibata, N., Ishitani, K., Kobayashi, M. and Ohta, H. (2011) Pentosidine accumulation in human oocytes and their correlation to age-related apoptosis. *Acta Histochem. Cytochem.* 44; 61–72.
- Matsuzawa, S. I. and Reed, J. C. (2001) Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses. *Mol. Cell.* 7; 915–926.
- McCluggage, W. G. and Young, R. H. (2006) Paraganglioma of the ovary: report of three cases of a rare ovarian neoplasm, including two exhibiting inhibin positivity. *Am. J. Surg. Pathol.* 30; 600–605.
- Miwa, N., Kobayashi, M., Takamatsu, K. and Kawamura, S. (1998) Purification and molecular cloning of a novel calcium binding protein, p26olf, in the frog olfactory epithelium. *Biochem. Biophys. Res. Commun.* 251; 860–867.
- Moore, B. W. (1965) A soluble protein characteristic of the nervous system. *Biochem. Biophys. Res. Commun.* 19; 739–744.
- Ødegaard, E., Davidson, B., Engh, V., Onsrud, M. and Staff, A. C. (2008) Assessment of endoglin and calprotectin as potential biomarkers in ovarian carcinoma and borderline tumors of the ovary. *Am. J. Obstet. Gynecol.* 199; 533. e1–8.
- Ohuchida, K., Mizumoto, K., Ishikawa, N., Fujii, K., Konomi, H., Nagai, E., Yamaguchi, K., Tsuneyoshi, M. and Tanaka, M. (2005) The role of S100A6 in pancreatic cancer development and its clinical implication as a diagnostic marker and therapeutic target. *Clin. Cancer Res.* 11; 7785–7793.
- Rammes, A., Roth, J., Goebeler, M., Klempt, M., Hartmann, M. and Sorg, C. (1997) Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. *J. Biol. Chem.* 272; 9496–9502.
- Robker, R. L. and Richards, J. S. (1998) Hormonal control of the cell cycle in ovarian cells: proliferation versus differentiation. *Biol. Reprod.* 59; 476–482.
- Sanders, S. L. and Stouffer, R. L. (1997) Localization of steroidogenic enzymes in macaque luteal tissue during the menstrual cycle and simulated early pregnancy: immunohistochemical evidence supporting the two-cell model for estrogen production in the primate corpus luteum. *Biol. Reprod.* 56; 1077–1087.
- Tinfo, N. and Komar, C. (2007) Potential role for peroxisome proliferator-activated receptor gamma in regulating luteal lifespan in the rat. *Reproduction* 133; 187–196.
- Tonini, G. P., Fabretti, G., Kuznicki, J., Massimo, L., Scaruffi, P., Brisigotti, M. and Mazzocco, K. (1995) Gene expression and protein localization of calcyclin, a calcium-binding protein of the S-100 family in fresh neuroblastomas. *Eur. J. Cancer* 31A; 499–504.