S100 proteins: An emerging cynosure in pregnancy & adverse reproductive outcome

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S100 proteins are calcium (Ca\(^{2+}\))-binding proteins and these have an important function in progression, manifestation and therapeutic aspects of various inflammatory, metabolic and neurodegenerative disorders. Based on their involvement in intracellular or extracellular regulatory effects, S100 proteins are classified into three subgroups: one subgroup is specialized in exerting only intracellular effects, other performs both intracellular and extracellular functions and the third subgroup members only display extracellular regulatory effects. S100 proteins are expressed particularly in vertebrates and have cell-specific expression. Functionally, S100 proteins act through their surface receptors and regulate cell functions in autocrine or paracrine mode. Receptor for advanced glycation end products (RAGEs) and toll-like receptor 4 are the main surface receptors. S100 proteins participate in the regulation of cellular differentiation, proliferation, apoptosis and inflammation along with Ca\(^{2+}\) homeostasis, energy metabolism and cellular migration, and perform the respective functions through their interaction with transcription factors, nucleic acids, enzymes, receptors, cytoskeleton system, etc. Currently, their role in adverse pregnancy outcomes and compromised reproductive health is being explored. These proteins are present in amniotic fluid, endometrium tissue and foetal brain; therefore, it is quite likely that alterations in the expression levels of S100 family members will be affecting the particular function they are involved in and ultimately affecting the pregnancy in adverse manner. The current review discusses about an association of S100 proteins in pregnancy disorders such as endometriosis, intrauterine growth retardation and miscarriage.

Key words Calcium signalling - early pregnancy loss - high-risk pregnancy - implantation - inflammation - intrauterine growth retardation

Introduction

Early miscarriage and pregnancy-associated problems are of major concern. The reason behind this is not only genetical or physiological but also environmental and modern lifestyle. Moderate levels of inflammatory reactions are also pre-requisite during the first trimester of pregnancy for implantation and embryo development. These early stages of pregnancy resemble ‘an open wound’\(^1\). For invasion and proper blood supply of embryo neovascularization and tissue remodelling occur during early gestational weeks of pregnancy\(^1,2\). An appropriate tuning of anti-inflammatory and inflammatory mediators is required for adequate repair of the uterine epithelium

\(^{#}\)Equal contribution
and the removal of cellular debris. Thus, this critical period of pregnancy is marked by expression of specific cytokines and adhesion molecules by both foetal and maternal side ensuring successful pregnancy. Any alteration and dysfunction of this balanced inflammatory milieu and any perturbation or disturbance in this during the critical period result in miscarriage or pregnancy-associated complications.3

Earlier studies in mice and human revealed the role of important calcium (Ca\(^{2+}\))-binding S100 proteins in pregnancy-related complications4,5. This group of proteins helps in the recruitment of leucocytes at inflammatory site and functions like cytokines6. These proteins regulate a variety of cellular functions such as cellular differentiation, cell cycle progression and energy intracellular signal transduction by interacting with several other mediatory proteins6. S100 proteins were found to be tumorigenic in function and get elevated in several cancer and melanoma cases6. An earlier study in human also showed elevated level of S100 group proteins in high-risk pregnancy cases, in amniotic fluid and cord blood of foetus with brain damage. The role of S100 protein in immunomodulation of high-risk pregnancy cases is an active area of research and clinical investigation. This review focuses on new advances regarding the role of S100 protein in diagnosis and treatment of high-risk pregnancies.

**S100 protein structure and function**

Ca\(^{2+}\) regulates several cellular processes and acts as a messenger. Many Ca\(^{2+}\)-binding proteins, having the EF-hand structural motif, make Ca\(^{2+}\) signalling network in combination with many molecular components. S100 proteins are the largest subgroup within this family of Ca\(^{2+}\)-binding proteins and found to be involved in several diseases such as rheumatoid arthritis, acute inflammatory lesions, cardiomyopathy, Alzheimer’s disease and cancer.6,10,11

S100 proteins are acidic, Ca\(^{2+}\)-binding proteins initially identified in the brain of several mammalian species and called S100 because of their solubility in 100 per cent ammonium sulphate.12,13 Genes responsible for the synthesis of most S100 proteins are located on human chromosomes 1q21.14 Initially, S100 proteins were found to be located in glial cells and used as a marker of glial cell differentiation and mammalian brain development.15-17 S100 protein family has 21 members having the same basic structural moiety but entirely different function, and are found in cerebrospinal fluid, urine, serum, seminal plasma and saliva mainly in active disease states. These proteins are found to be present in Ca\(^{2+}\) free (apo); Ca\(^{2+}\)-bound and target bound states as a symmetric dimer, with each monomer containing two EF-hand motifs.18 The EF-hand motif on N-terminal site contains helix I with pseudo Ca\(^{2+}\)-binding site, and the EF-hand of C-terminal is associated with helix III, helix IV and second Ca\(^{2+}\)-binding site (Fig. 1).

S100 proteins undergo structural and conformational changes on binding with Ca\(^{2+}\), and this conformational change allows interaction of these proteins with target molecules. Activated S100 proteins perform all cellular functions by both extracellular and intracellular methods (Table 1). All S100 proteins function in the form of dimmers, and only S100G protein acts as monomer. A few hetero-dimers are also reported: S100A1/B, S100A8/A9, S100A1/A4 and S100A1/P. S100 proteins can also form active tetramers, hexamers or larger oligomers (S100B, S100A4, S100A8/A9 and S100A12).

**S100 receptors**

Function of S100 proteins is determined by their oligomeric forms and their respective binding partners.46 Extracellular S100 proteins act via activation of surface receptors such as G protein-coupled receptors, receptor for advanced glycation end products (RAGEs) and toll-like receptors and aid in regulatory processes such as cell proliferation, differentiation and migration in normal as well as different pathological conditions. Intracellular S100 proteins also act via interaction with different target enzymes, cytoskeleton subunits, receptors and transcription factors or nucleic acids regulate Ca\(^{2+}\) homeostasis, energy metabolism and cellular differentiation.

**Role of S100 proteins in high-risk pregnancy cases**

In maternal endometrium, S100 proteins are expressed by both immune cells and non-immune cells. A few groups of S100 proteins such as S100A8, S100A9 and S100A12 are mainly secreted from myeloid origin of immune cells such as granulocytes, monocytes and early stages of macrophages. As myeloid origin cells are well known as crucial regulators for other immune cells (T, Treg, uNK and non-inflammatory macrophages and neutrophils cells) in successful pregnancy, any alteration in inflammatory or immunomodulatory stage may change S100 protein levels (Fig. 2). Some non-immune
S100A8 protein recruits mouse and human neutrophils and macrophages at the site of inflammation. Endometrial epithelium and stromal cells also showed expression of S100A10 protein during the implantation window and found to play an important role in endometrial receptivity. The expression of these proteins have been found to be down regulated in the endometrium of infertile patients. This is the reason behind the failure of 30 per cent of embryo implantation in assisted reproduction.

S100 proteins regulate embryo implantation, intrauterine growth and normal foetal brain development during pregnancy. S100 family proteins have been found to be dysregulated in various endometrial diseases (Table II). S100A8 proteins are found to be down regulated in receptive phase of endometrium. S100A8 protein recruits mouse and human neutrophils and macrophages at the site of inflammation. Endometrial epithelium and stromal cells also showed expression of S100A10 protein during the implantation window and found to play an important role in endometrial receptivity. The expression of these proteins have been found to be down regulated in the endometrium of infertile patients. This is the reason behind the failure of 30 per cent of embryo implantation in assisted reproduction.
S100 A1: Skeletal system, neurons and cardiomyocytes
S100 A2: Cancerous cells
S100 A3: Localized in root of hair and some cancerous astrocytes
S100 A4: Tumorous tissue
S100 A5: Tumorous tissue
S100 A6: Tumorous tissue
S100 A7: Tumorous tissue
S100 A8: Macrophages, dendritic cells, microvascular endothelial cells
S100 A9: Neutrophils, dendritic cells
S100 A10: Neutrophils, dendritic cells
S100 A11: Neutrophils, dendritic cells
S100 A12: In neutrophils and inducible in macrophages
S100 A13: Fibroblasts, osteoblasts and melanoma cells
S100 A14: Tumorous tissue
S100 A15: -
S100 A16: Tumorous tissue
S100 B: Astrocytes, certain neuronal populations, Schwann cells, melanocytes, chondrocytes, adipocytes, skeletal myofibers
S100 G: Neutrophils, dendritic cells
S100 P: Tumorous tissue
S100 Z: Tumorous tissue

VSCM, vascular smooth muscle cell; FGF, fibroblast growth factor; IL, interleukin
**Table II.** Altered expression profile of S100 proteins in various human pregnancy-related diseases

| Pregnancy-related diseases                        | S100 family | References |
|--------------------------------------------------|-------------|------------|
| Pregnancy-associated with Down syndrome          | Upregulated S100B | 49         |
| IUGR                                             | Upregulated S100B | 50         |
| SGA babies SGA foetus                            | No change in S100B | 51         |
| Pre-eclampsia+IUGR                               | Upregulated S100B | 52         |
| Miscarriage                                      | Down regulated S100A11, Up regulated S100A8, S100A9 | 53, 4,47   |
| Pre-eclampsia                                    | Up regulated S100B | 52         |
| Pre-term labour                                  | Up regulated S100B | 51         |
| IVF failure                                      | Down regulated S100A11, S100A10 | 54         |
| PCOS                                             | Up regulated S100A12 | 55         |
| Endometriosis                                    | Up regulated S100A4 | 56         |
| Endometriosis associated with infertility        | Up regulated S100P | 56         |
| Gestational diabetes                             | Up regulated S100A9 | 57         |

IUGR, intrauterine growth restriction; SGA, small for gestational age; IVF, *in vitro* fertilization; PCOS, polycystic ovary syndrome.

**Fig. 2.** Schematic diagram represents interaction of S100 proteins with immune cells for the regulation of various hallmark processes of pregnancy. IFN-γ, interferon gamma; IL, interleukin; TH, T helper; TNF-α, tumor necrosis factor alpha; uNK, uterine natural killer. Source: Refs 4, 47.

**Conclusion**

The present review summarizes the role of S100 proteins in high-risk pregnancy cases along with its structure and mechanism of action. This also covers the importance of S100 proteins as a main player of successful implantation, embryonic growth and birth of physically and mentally healthy child. The optimal expression and signalling of S100 proteins, at particular stages of pregnancy is a pre-requisite for avoiding high-risk pregnancy cases and can serve as therapeutic target and prognostic biomarker in pregnancy-related complications.

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**References**

1. Mor G, Cardenas I. The immune system in pregnancy: A unique complexity. *Am J Reprod Immunol* 2010; 63: 425-33.

2. Mor G, Cardenas I, Abrahams V, Guller S. Inflammation and pregnancy: The role of the immune system at the implantation site. *Ann N Y Acad Sci* 2011; 1221: 80-7.
3. Kwak-Kim J, Yang KM, Gilman-Sachs A. Recurrent pregnancy loss: A disease of inflammation and coagulation. J Obstet Gynaecol Res 2009; 35: 609-22.

4. Nair RR, Khanna A, Singh K. Association of increased S100A8 serum protein with early pregnancy loss. Am J Reprod Immunol 2015; 73: 91-4.

5. Passey RJ, Williams E, Lichanska AM, Wells C, Hu S, Geczy CL, et al. Null mutation in the inflammation-associated S100 protein S100A8 causes early resorption of the mouse embryo. J Immunol 1999; 163: 2209-16.

6. Kanamori T, Takakura K, Mandai M, Kariya M, Fukuhara K, Sakaguchi M, et al. Increased expression of calcium-binding protein S100 in human uterine smooth muscle tumours. Mol Hum Reprod 2004; 10: 735-42.

7. Michetti F, Gazzolo D. S100B testing in pregnancy. Clin Chim Acta 2003; 335: 1-7.

8. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 2000; 1: 11-21.

9. Kawasaki H, Nakayama S, Kretsinger RH. Classification and universality of calcium-binding proteins of the EF-hand type with intracellular and extracellular functional roles. J Biol Chem 2001; 276: 398-403.

10. Heizmann CW, Cox JA. New perspectives on S100 proteins: A multi-functional Ca(2+)-, Zn(2+)- and Cu(2+)-binding protein family. Biometals 1998; 11: 277-95.

11. Kessler D, Levine L, Fasman G. Some conformational changes in CNS levels of the S-100 and 14-3-2 proteins during development and aging of the mouse. Biochim Biophys Acta 1998; 1223: 383-97.

12. Kessler D, Levine L, Faesman G. Some conformational and immunological properties of a bovine brain acidic protein (S-100). Biochemistry 1968; 7: 758-64.

13. Moore BW. A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 1965; 19: 739-44.

14. Heizmann CW, Fritz G, Schäfer BW. S100 proteins: Structure, functions and pathology. Front Biosci 2002; 7: d1356-68.

15. Ciceri TJ, Ferrendelli JA, Suntzeff V, Moore BW. Regional changes in CNS levels of the S-100 and 14-3-2 proteins during development and aging of the mouse. J Neurochem 1972; 19: 2119-25.

16. De Vitry F, Picart R, Jacque C, Legault L, Dupouey P, Tixier-Vidal A, et al. Presumptive common precursor for neuronal and glial cell lineages in mouse hypothalamus. Proc Natl Acad Sci U S A 1980; 77: 4165-9.

17. Herschman HR, Levine L, De Vellis J. Appearance of a brain-specific antigen (S-100 protein) in the developing rat brain. J Neurochem 1971; 18: 629-33.

18. Santamaria-Kisiel L, Rintala-Dempsey AC, Shaw GS. Calcium-dependent and -independent interactions of the S100 protein family. Biochem J 2006; 396: 201-14.

19. Donato R. S100: A multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 2001; 33: 637-68.

20. Wolf S, Haase-Kohn C, Pietzsch J. S100A2 in cancerogenesis: A friend or a foe? Amino Acids 2011; 41: 849-61.
moporphino antisense oligonucleotides. Proc Natl Acad Sci U S A 2004; 101 : 8028-33.

36. Austermann J, Nazmi AR, Müller-Tidow C, Gerke V. Characterization of the Ca2+ -regulated ezrin-S100P interaction and its role in tumor cell migration. J Biol Chem 2008; 283 : 29331-40.

37. Grebienko AV, Hopper JE, Makhatadze GI. Molecular characterization and tissue distribution of a novel member of the S100 family of EF-hand proteins. Biochemistry 2001; 40 : 15538-48.

38. Bresnick AR, Weber DJ, Zimmer DB. S100 proteins in cancer. Nat Rev Cancer 2015; 15 : 96-109.

39. Skelton NJ, Kördel J, Akke M, Forsén S, Chazin WJ. Signal transduction versus buffering activity in Ca2+-binding proteins. Nat Struct Mol Biol 1994; 1 : 239.

40. Wang G, Zhang S, Fernig DG, Spiller D, Martin-Fernandez M, Zhang H, et al. Heterodimeric interaction and interfaces of S100A1 and S100P. Biochem J 2004; 382 : 375-83.

41. Lügering N, Stoll R, Schmid KW, Kucharzik T, Stein H, Burmeister G, et al. The myeloic related protein MRP8/14 (27E10 antigen) – Usefulness as a potential marker for disease activity in ulcerative colitis and putative biological function. Eur J Clin Invest 1995; 25 : 659-64.

42. Ostendorp T, Leclerc E, Galichet A, Koch M, Demling N, Weigle B, et al. Structural and functional insights into RAGE activation by multimeric S100B. EMBO J 2007; 26 : 3868-78.

43. Kiryushko D, Novitskaya V, Soroka V, Klingelhofer J, Lukandin E, Berezin V, et al. Molecular mechanisms of Ca(2+) signaling in neurons induced by the S100A4 protein. Mol Cell Biol 2006; 26 : 3625-38.

44. Leukert N, Vogl T, Strupat K, Reichelt R, Sorg C, Roth J, et al. Calcium-dependent tetramer formation of S100A8 and S100A9 is essential for biological activity. J Mol Biol 2006; 359 : 961-72.

45. Moroz OV, Anson AA, Dodson EJ, Burrell HJ, Grist SJ, Lloyd RM, et al. The structure of S100A12 in a hexameric form and its proposed role in receptor signalling. Acta Crystallogr D Biol Crystallogr 2002; 58 : 407-13.

46. Desamero MJ, Delgado RA, de Ocampo GD, Baruian JV, Collantes TM, Estacio MA. Immunohistochemical demonstration of S100 protein in the ovary of the Philippine water buffalo (Bubalus bubalis Linnaeus, 1758) (Artiodactyla: Bovidae). Philipp J Vet Med 2015; 52.

47. Nair RR, Khanna A, Singh K. Role of inflammatory proteins S100A8 and S100A9 in pathophysiology of recurrent early pregnancy loss. Placenta 2013; 34 : 824-7.

48. Fritz G, Botelho HM, Morozova-Roche LA, Gomes CM. Natural and amyloid self-assembly of S100 proteins: Structural basis of functional diversity. FEBS J 2010; 277 : 4578-90.

49. Abrah HD, Noble PL, Nicolaides KH, Sherwood RA. Maternal serum S100 protein in normal and down syndrome pregnancies. Prenat Diagn 1999; 19 : 334-6.

50. Gazzolo D, Marinoni E, di Iorio R, Lituania M, Bruschettini PL, Michetti F, et al. Circulating S100beta protein is increased in intrauterine growth-retarded fetuses. Pediatr Res 2002; 51 : 215-9.

51. Morales-Roselló J, Khalil A, Alba-Redondo A, Martinez-Triguero L, Akhoundova F, Perales-Marin A, et al. Protein S100β in late-pregnancy fetuses with low birth weight and abnormal cerebroplacental ratio. Fetal Diagn Ther 2017; 41 : 15-22.

52. Tskitishvili E, Komoto Y, Temma-Asano K, Hayashi S, Kinugasa Y, Tsubouchi H, et al. S100B protein expression in the amnion and amniotic fluid in pregnancies complicated by pre-eclampsia. Mol Hum Reprod 2006; 12 : 755-61.

53. Liu AX, Jin F, Zhang WW, Zhou TH, Zhou Y, Yao WM, et al. Proteomic analysis on the alteration of protein expression in the placental villous tissue of early pregnancy loss. Biol Reprod 2006; 75 : 414-20.

54. Liu XM, Ding GL, Jiang Y, Pan HJ, Zhang D, Wang TT, et al. Down-regulation of S100A11, a calcium-binding protein, in human endometrium may cause reproductive failure. J Clin Endocrinol Metab 2012; 97 : 3672-83.

55. Su NJ, Ma J, Feng DF, Zhou S, Li ZT, Zhou WP, et al. The peripheral blood transcriptome identifies dysregulation of inflammatory response genes in polycystic ovary syndrome. Gynecol Endocrinol 2018; 34 : 584-8.

56. Hapangama DK, Raju RS, Valentijn AJ, Barracough D, Hart A, Turner MA, et al. Aberrant expression of metastasis-inducing proteins in ectopic and matched eutopic endometrium of women with endometriosis: Implications for the pathogenesis of endometriosis. Hum Reprod 2012; 27 : 394-407.

57. Oliva K, Barker G, Rice GE, Bailey MJ, Lappas M. 2D-DIGE to identify proteins associated with gestational diabetes in omental adipose tissue. J Endocrinol 2013; 218 : 165-78.

58. Portela LC, Tort AB, Neto EC, Kessler RG, Penchaszadeh V, Souza DO, et al. High immunocontent of S100 beta protein in amniotic fluid of pregnancies complicated by pre-eclampsia. PLoS One 2013; 8 : e58419.