ROLE OF SECRETED PHOSPHOPROTEIN 1 (SPP1) GENE IN BOVINEN- A REVIEW

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ABSTRACT

Secreted phosphoprotein 1 (SPP1) popularly known as osteopontin (OPN) is a highly phosphorylated glycoprotein that is a prominent component of the mineralized extracellular matrices of bones and teeth. It is found in milk, plasma, urine and expressed in several tissues. Comparative sequence analysis of the bovine OPN cDNA in various species has revealed both conserved and non-conserved sequences. This gene is located in BTA 6 and having 7 exons. SPP1 gene has roles in bone mineralization, cancer metastasis, cell-mediated immune responses, inflammation and cell attachment. Polymorphisms in cattle and buffaloes were reported by various workers. SPP1 gene has potent roles in growth, production and reproduction of the animals. This gene is said to be associated with various traits like milk yield and milk compositions, growth and body weight, stillbirth and dystocia, mastitis and infections in bovine. OPN is reported to upregulate and promote the expression and secretion of Th1 cytokines. SPP1 variants are associated with mastitis resistance. SPP1 is involved in muscle regeneration after injury. There is increase in SPP1 expression in subclinical cows compared to control cows in Johne’s disease. OPN has an important anti-apoptotic factor in many circumstances. As SPP1 gene is a good candidate gene for traits like stillbirth, dystocia, protein percentage of milk, twinning rate etc, it has great potential to be used in marker assisted selection and hence in improving genetic progress in cattle breeding.

Key Words : Secreted phosphoprotein 1 (SPP1 gene), Osteopontin(OPN gene), Polymorphism, Association, Stillbirth, Dystocia

Secreted phosphoprotein 1 (SPP1) is a glycoprotein member of the small integrin binding ligand, N-linked glycoprotein (SIBLING) family of genetically related extracellular matrix (ECM) proteins. It is a multifunctional matricellular protein (Matricellular proteins are unique ECM molecules that do not appear to have direct structural roles but instead mediate cell-matrix interaction and cell function.). SPP1 was identified independently, together with bone sialoprotein (BSP), as a major sialoprotein in the extracellular matrix of bone (Zhang et al., 1990) and the 2 proteins initially called bone sialoprotein (BSP I) and bone sialoprotein II (BSP II), respectively (Franzen and Heinegard, 1985). Secreted phosphoprotein (SPP1) is also known as osteopontin protein (OPN), bone sialoprotein I (BSP-1), early T-lymphocyte activation (ETA-1)

1National Bureau of Animal Genetic Resources, Karnal- 132 001, India
and Osteopontin-K. SPP1 was first identified in 1986 in osteoblasts. The name “osteonpontin” was introduced to reflect the potential of the bone protein to serve as a bridge between cells and hydroxyapatite through RGD and polyaspartic acid motifs discovered in the primary sequence of the protein (Oldberg et al., 1986). The prefix of the word “osteo” indicates that the protein is expressed in bone although it is also expressed in other tissues. OPN is characterized by the presence of a polyaspartic acid sequence and sites of Ser/Thr phosphorylation that mediate hydroxyapatite binding motif that mediates cell attachment and signaling (Sodek et al., 2000). SPP1 is produced by both immune and nonimmune cells such as osteoclasts, smooth muscle cells and epithelial cells (Denhardt and Guo, 1993). The primary immune sources of OPN are activated macrophages (Atkins et al., 1998) and activated T cells (Ashkar et al., 2000). The protein is also found in milk, plasma and urine. Osteopontin had been detected in raw milk of cows at a concentration of 8 mg/L (Bayless et al., 1997).

1. **Position and general description about the gene**

Osteopontin gene exhibits a moderate level of sequence conservation (Crivello and Delvin, 1992). Comparative sequence analysis of the bovine OPN cDNA in various species has revealed both conserved and non-conserved sequences (Kerr et al., 1991). It was found, for example, that the bovine and ovine sequences have a 22-AA gap compared with all other examined species (Leonard et al., 2005). Several whole-genome scans in cattle have identified QTL affecting milk production traits on BTA 6 close to OPN gene location (Zhang et al., 1998; Nadesalingam et al., 2001; Ron et al., 2001; Ashwell et al., 2004; Olsen et al., 2004). Olsen et al. (2005) positioned a QTL affecting milk production traits to an interval of 420 kb between the genes ABCG2 [ATP binding cassette, subfamily G (WHITE), member 2] and LAP3 (leucine aminopeptidase 3) on chromosome 6 in bovines. This narrow region harbors only 6 genes, including OPN. The SPP1 is highly negative charged, extracellular matrix protein that lacks an extensive secondary structure. There are seven exons in this gene (Figure 1). The exons are 86, 68, 39, 81, 42, 303 and 748 bp in length. The gene has been mapped to 4q13 in the human genome (Young et al., 1990) and to the ric' gene on mouse chromosome 5 (Fet et al., 1989; Pataraca et al., 1989). The gene is located on chromosome 8 in pig (Denhardt and Guo, 1993). It is composed of about 300 amino acids (294 in Mus musculus, 314 in Homo sapiens, 278 in Bos Taurus, 280 in Bubalus bubalis and 303 in Sus scrofa). Tantia et al. (2008) compared bubaline OPN with taurine OPN. They found 12 nucleotide differences in the exonic region (two in exon 1, seven in exon 6 and three in exon 7). There was complete homology in exons 2–5. The bubaline OPN had insertion of two amino acids at positions 94 (Aspartic acid) and 227 (Asparagine), making a total of 280 amino acids.

![NC_007304.3](image-url)  
*Fig. 1*: SPP1 Gene.
2. Biological functions of SPP1

Osteopontin (SPP1, OPN, and Eta-1) is a secreted glycoprotein that plays a role in many different mammalian biological functions. It has been shown to be involved in bone mineralization, cancer metastasis, cell-mediated immune responses, inflammation, cell survival, interactions with Ca\(^{++}\) ions, embryo implantation and maintenance of pregnancy and angiogenesis (Denhardt and Guo, 1993; Butler et al., 1996; Weber and Cantor, 1996; Giachelli and Steitz, 2000; Denhardt et al., 2001). The immunological roles of OPN is presented in Figure 2.

OPN contains a functional Gly-Arg-Gly-Asp-Ser (GRGDS) cell-binding domain (Oldberg et al., 1986). This domain is present in cell adhesion molecules and is involved in cell attachment and spreading reactions in vitro via integrins (Hynes, 1987). Integrins are a large family of cell adhesion receptors that mediate a number of diverse functions, including cell–matrix and cell–cell interactions (Hynes, 1987; Vinatier, 1995). SPP1 has potential to influence tissue remodeling at the conceptus–maternal interface by affecting cell–cell and cell–ECM communication, increasing cell proliferation, migration and survival, and regulating local cytokine networks (Johnson et al., 2003).

The role of SPP1 in growth pathways has been studied extensively in bone tissue growth and cancer progression (Standal et al., 2004; Rangaswami et al., 2006). SPP1 has been shown to increase in a myoblast proliferation and differentiation in vitro model (Ishibashi et al., 2005). OPN knockout mice did not loose bone in a model of hind-limb disuse (tail suspension), showing the importance of OPN in bone remodeling in an experiment carried out by Malval et al., (2008). The role of OPN for the osteoclast formation is presented in Figure 3 (Denhardt, and Guo 1993).

Nagatomo et al. (2004) reported that both mRNA and protein levels were highly expressed during lactation and thus seem to have an effect on milk production traits. The role of OPN in mammary gland development and lactation has been confirmed using a transgenic mouse model expressing OPN antisense RNA in the mammary gland (Nemir et al., 2000). The presence of OPN in milk and the high

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**Fig 2**: Immunologic functions of OPN. OPN binds to several integrin receptors including $\alpha_4\beta_1$, $\alpha_9\beta_1$ and $\alpha_9\beta_4$ expressed by leukocytes and are known to induce cell adhesion, migration, and survival in immune cells including neutrophils, macrophages, T cells, mast cells, and osteoclasts. (http://en.wikipedia.org/wiki/Osteopontin)
expression in mammary gland epithelial cells may account for the proliferation and differentiation of mammary glands (Nagatomo et al., 2004). Schnabel et al. (2005) proposed SPP1 as a candidate gene, because this gene has an essential role in mammary gland differentiation and branching of the mammary epithelial ductal system. OPN blocks the activation induced cell death of macrophages, T cells, fibroblasts and endothelial cells exposed to harmful stimuli (Denhardt et al., 2001; Standal et al., 2004). OPN prevents non-programmed cell death in inflammatory colitis (Da Silva et al., 2006).

Evidence of altered growth has been shown in SPP1 knockout mice by examining embryo size in utero at 3 stages of gestation using magnetic resonance microscopy. Homozygous knockout mice had smaller embryos at all 3 stages of gestation with no differences in litter size when compared with the controls (Weintraub et al., 2004). SPP1 is involved in muscle regeneration after injury (Goetsch et al., 2003). Siiteri et al. (1995) detected mRNA for OPN in sertoli cells, pachytene spermatocytes or round spermatocytes by RT-PCR studies in mouse testis.

3. Polymorphisms in SPP1 gene in cattle

Using 38 microsatellite markers in a pedigree of 3,147 Holstein bulls Schnabel et al. (2005) fine mapped regions of BTA6 and sequenced a 12.3-kb region harboring Osteopontin, a positional candidate for the statistically most significant of the identified QTL. They reported nine mutations and only genotypes for the OPN3907 indel were concordant with the QTL genotypes of eight bulls that were established by segregation analysis. An SNP that was believed to be a regulatory region of the SPP1 gene had been proposed as a causative mutation affecting milk yield, fat yield, fat percent, and protein percent in dairy cattle. A single nucleotide polymorphism in introns 4 (C/T) was detected and primers were designed to amplify genomic DNA from 1362 bulls obtained from Cooperative Dairy DNA Repository (CDDR) and from 214 cows from the herd of University of Wisconsin (UW) in United States (Leonard et al., 2005).

The polymorphism in SPP1 termed OPN3907 was an insertion/deletion unsuitable for genotyping by mass spectrometry of an extension assay and was instead assayed as a length polymorphism on a LI-COR 4200 DNA Analysis System (LICOR Biosciences, Lincoln, NE) (White et al., 2007).

Fig.3: Roles OPN might play in osteoclast function. A) OPN promotes attachment of osteoclasts to the bone surface via interactions on the one hand with the hydroxyapatite crystal, and on the other hand with the cell surface, possibly the α3β1 integrin. Proteins that OPN may interact include osteocalcin, type I collagen, and fibronectin. OPN may also contribute to isolating the absorption lacuna from the external milieu. B) OPN promotes changes in gene expression via a signal transduction cascade initiated by an interaction of OPN with a cell surface receptor such as the α3β1 integrin. (Denhardt, and Guo 1993)
observed alleles consisted of 9 or 10 consecutive thymidine base pairs on the sense strand and an indel polymorphism (T_9/T_10) was reported to be highly associated with beef production traits (White et al., 2007).

Khatib et al. (2007) reported the additive effects of SPP1 C>T polymorphism in 931 samples of in Holstein cattle significantly associated with fat percentage, protein percentage and fat yield.

High SPP1 polymorphism was observed in panel animals of Hereford (n=23), Limousine (n=24), Polish Holstein-Fresian (n=28) and Polish Red (n=23) cattle (Pareek et al., 2008) (Table 1). C>T SNP within the intron IV of SPP1 could be studied for trait association with muscle growth, body composition trait and milk production traits (Pareek et al., 2008). Oztabak et al. (2008) reported C>T SNP in intron 4 in South and East Anatolian Red cattle (Table 1).

4. Polymorphisms in buffaloes

Tantia et al. (2008) characterized the OPN in buffalo (Bubalus bubalis) and sequenced OPN gene from the genomic DNA as well as from the cDNA of buffalo mammary gland. A comparative analysis of the buffalo OPN gene and the presumed protein sequence with B. taurus as reference sequence (AY878328) was studied. They observed polymorphisms in 24 sequences of OPN gene among six different breeds of buffalo. A total of six SNPs were detected, five in intronic and one in the upstream region. The SNP in the upstream region (indel) of buffalo OPN was the same as reported by Schnabel et al. (2005) in B. taurus. All the SNPs in the intronic region were transitions.

5. Association of SPP1 with different traits

SPP1 gene is associated with various traits like milk yield and milk compositions, growth and body weight, stillbirth and dystocia, mastitis and infections.

5.1 Association with milk yield and milk composition

Significant association of OPN variants with milk composition traits were reported by Cohen et al. (2004), Leonard et al. (2005), Schnabel et al. (2005) and Khatib et al. (2007). Cohen et al. (2004) investigated the region of the microsatellite BM143 in the middle of chromosome 6 and milk composition in 420 Holstein bulls and reported that OPN gene had the highest linkage disequilibrium effects on protein percentage among 12 genes under that study. They concluded that OPN was the key candidate gene affecting milk protein percentage.

The SPP1 C>T SNP marker was first reported by Leonard et al. (2005) in a trait-associated study with milk production. Weighted least squares analysis was employed to study the effects of OPN variants on production and functional traits in both the Cooperative Dairy DNA Repository (CDDR) and

<table>
<thead>
<tr>
<th>Gene</th>
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<tr>
<td>SPP1</td>
<td>A &gt; G</td>
<td>Intron 5 (AJ871176)</td>
<td>Cohen-Zinder et al., 2005</td>
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<td></td>
<td>T &gt; G</td>
<td>Exon 7 (AJ871176)</td>
<td>Cohen-Zinder et al., 2005</td>
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<td>6 SNPs</td>
<td>5 kb upstream</td>
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<td>C &gt; T</td>
<td>Intron 4</td>
<td>Leonard et al., 2005</td>
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<td>9 SNPs</td>
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<td>Schnabel et al., 2005</td>
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<td>T_9/T_10 (in-del polymorphism)</td>
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University of Wisconsin (UW) herd populations. For the CDDR population, the C allele was associated with an increase in milk protein percentage \((P = 0.0255)\) and milk fat percentage \((P = 0.0480)\). The OPN variants did not show significant effects on milk, fat, or protein yields or SCS. Although not statistically significant, allele C showed a negative effect on milk yield because of the negative correlation \((-0.40)\) between this trait and milk protein percentage.

Schnabel et al. (2005) identified 6 SNPs in a region about 5 kb upstream of the bovine OPN, of which 1 SNP (a deletion/insertion) showed significant association with milk protein percentage. The OPN SNPs in the marker map increased the statistical support for the MY, FP, and PP QTL directly over OPN.

Tantia et al. (2008) reported no significant effect \((P \text{ value:} 0.1901)\) of the genotypes \((T_9/T_{10})\) on the milk yield in buffaloes.

5.2 Association with growth and body weight

The SPP1 marker was associated with yearling weight \((P = 0.025)\), live weight at slaughter \((P = 0.016)\), post weaning ADG \((P = 0.007)\), and hot carcass weight \((HCW) \(P = 0.007)\) in a large, multi-sire population representing the 7 most populous beef breeds in the United States (Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin, and Charolais) and post-weaning growth trait associations were confirmed in an independent population including sires from tropically adapted breeds (White et al., 2007). The SPP1 marker was associated with yearling weight \((P = 0.034)\), live weight at slaughter \((P = 0.011)\), and post-weaning ADG \((P = 0.015)\) and showed a trend toward association with hot carcass weight \((HCW) \(P = 0.083)\) in the populations studied. Overall, SPP1 showed a large, consistently observed effect on post-weaning growth in a variety of populations. The presence or absence of a single T9 allele resulted in the differences observed in the 2 populations were studied. The SPP1 polymorphism was consistently associated with effects on post-weaning growth traits in both populations. Additional trait associations for SPP1 marker were observed only in 1 of the 2 populations, limiting support for use of the marker to select for other traits (White et al., 2007).

The significant associations of the SPP1 marker with birth weight \((P < 0.006)\), weaning weight \((P < 0.007)\) were found in 12 breeds maintained in US Meat Animal Research Centre (Allan et al., 2007).

5.3 Association with stillbirth and dystocia

A genome wide association study by Olsen et al. (2009) for one million records of dystocia and stillbirth in Norwegian Red breed cattle suggested that SPP1 gene is a candidate gene and associated with stillbirth and dystocia in cattle. They reported that the region around marker number 285 (BTA-75776) which contains six known genes (SPP1, IBSP, MEPE, LAP3, MED28 and NCAPG) was the most significant position obtained by LDLA for Dystocia direct effect \((DYS_{dir})\).

5.4 Association with mastitis

The osteopontin transcript \((SPP1)\) was identified in the somatic cells from cows experimentally infected with Escherichia coli (Alain et al., 2009). They found four DNA polymorphisms in the SPP1 genomic sequence in bulls with extreme estimated breeding values (EBVs) for somatic cell scores (SCS). Somatic cell score is an indicator of mastitis. Statistical analysis revealed that the SNP SPP1c.-1301G>A had an impact on EBV for SCS \((P < 0.001)\). Using an allele substitution model, SPP1c.-1251C>T, SPP1c.-30G>A, and SPP1c.*40A>C had an impact on SCS. Analysis revealed statistically significant differences between haplotype groups at a comparison-wise level with sire EBVS for SCS for the first \((P = 0.012)\), second \((P < 0.001)\), and third \((P < 0.001)\) lactations. These reports were the link between DNA polymorphisms of SPP1, the number of milk immune cells and the susceptibility to mastitis. These SNPs were identified by in silico search in transcription factor recognition
sites which factors were presumably involved in the Th1 immune response and in the Th2 regulation pathway.

5.5 Association with bull reproductive tract and fertility

Rodriguez et al. (2000) examined gene expression of OPN in the bull reproductive tract for the first time. In bulls, OPN may protect the epithelial cells from bacterial infections in the accessory sex glands. OPN transcripts were not observed in the testis by Northern blot analysis, but transcripts were detected in the developing germ cells within the adluminal compartment of the seminiferous tubules by in situ hybridization (Rodriguez et al., 2000).

Considering the role of OPN as a cell adhesion molecule and the importance of cell-cell communication within the testis, it is possible that OPN may play a role in the adhesion of the developing germ cells to the Sertoli cells, the adhesion to other germ cells, or both. OPN may also be involved in the transfer of information between cells in the seminiferous tubules (Rodriguez et al., 2000).

Cancel et al. (1999) were unable to detect OPN protein in the bull testis or on ejaculated spermatozoa using an anti-OPN polyclonal antibody. Despite the inability to detect the OPN protein on bull sperm, OPN transcripts were detected in the germ cells within the seminiferous tubules, epididymis and ampulla (Rodriguez et al., 2000). Some laboratories have demonstrated the presence of specific mRNA in the nuclei of mature spermatozoa. Messenger RNAs for the protamines PRM-1 and PRM-2, and the transition protein TP-1 (Wykes et al., 1997), as well as beta-actin, cMYC, and HLA1 (Miller, 1997) have been reported. Even though osteopontin was reported to influence bull fertility (Cancel et al., 1997; Erikson et al., 2007), Alain et al. (2009) did not find significant statistical association of the four SPP1 SNP with the EBV of the non-return rate which is associated with male fertility.

5.6 Association with infections

Bacterial strains that are capable of binding to epithelial cells via integrins may not be able to attach to the epithelium due to the presence of OPN that also compete for integrin-binding domains present on the epithelial cells (Brown et al., 1992).

OPN is reported to upregulate and promote the expression and secretion of Th1 cytokines (Karcher et al., 2008). When Opn knockout mice were challenged with Mycobacterium bovis bacillus Calmette-Guerin (BCG Pasteur), they had more severe infection, heavier bacterial loads, and greater granuloma burdens compared with the wild-type mice (Nau et al., 1999).

The ability of OPN to promote a Th1 immune response and increase resistance to mycobacterial infections makes this cytokine important to study in Mycobacterium avium spp. paratuberculosis (MAP) -infected cattle. An effective Th1 response to MAP infection is critical for controlling the initial stages of the disease.

Subclinical Johne’s disease (JD) cows produce greater amounts of the Th1 cytokines IFN-γ and TNF-α compared with clinical cows (Stabel, 2000). There was an increase in OPN expression in subclinical cows compared with control and clinical cows. That was important, because an increase in OPN expression supports the paradigm for strong Th1 host responses in subclinically infected dairy cows (Karcher et al., 2008). Increased OPN production by this cell type would promote the activation of macrophages, which in turn would induce the secretion of IL-12. Thus, creating a positive feedback loop to enhance the cell-mediated immune response typical to what is observed in subclinically infected cows. Osteopontin has been

Three OPN isomers at 60, 40, and 22 kDa were identified in the testicular parenchyma of the bull reproductive tract (Erikson et al., 2007).
shown to enhance the production of Th1 cytokines, including IL-12 and TNF-α (Ashkar et al., 2000; Weber et al., 2002).

Previous reports have demonstrated that subclinical Johne’s disease (JD) cows have greater expression and secretion of IFN-γ, another Th1 cytokine, compared with that of clinical cows (Stabel, 2000; Khalifeh and Stabel, 2004). Along with IFN-γ, TNF-α is involved in the early stages of mycobacterial infections by controlling bacterial proliferation (Appelberg, 1994).

There was a positive correlation between OPN expression and the expression of the Th1 cytokines IL-12 and IFN-γ in clinically infected cattle by Pearson correlation method (Karcher et al., 2008).

A study reported the ability of Opn knockout mice to respond to an experimental infection with the influenza virus and vaccinia virus (Abel et al., 2005).

Osteopontin is also associated with cholesterol gallstone formation in human and mouse (Ichikawa et al., 2009).

CONCLUSION

Secreted phosphoprotein 1 (SPP1) is one of the most important glycoprotein of SIBLING family. The SPP1 gene has several biological functions in animals. It is a multifunctional matricellular protein. It controls growth, production, reproduction and immunity of the animals. Although some studies suggested that OPN itself affects milk protein percentage, further investigation of the OPN gene, including upstream and downstream control regions, is needed to explore molecular mechanisms causing the QTL effects. The mechanism of SPP1 affecting fat yield and fat percentage in bovine milk is still unknown. OPN can also be used as a tool for diversity analysis in buffaloes. The presence of OPN mRNAs in spermatozoa suggests its potential roles in reproduction.

So, further studies may be carried out for role of SPP1 genes for other traits such as longevity and fertility. The genetic potential of OPN gene variants in terms of selection for the improvement of mastitis resistance in dairy cows, will be beneficial for dairy farmers. There is need for future experiments to further understand the mechanism of OPN in the bovine immune system.

SPP1 gene is a good candidate gene for many traits (Stillbirth and dystocia, protein percentage of milk, twinning rate etc). So, it has great potential to be used in marker assisted selection and hence in improving genetic progress in cattle breeding.

REFERENCES

http://en.wikipedia.org/wiki/Osteopontin