GENERAL INTRODUCTION

1. Sperm protein 17 (Sp17) background

Sperm protein 17 (Sp17) is a presumed testes-specific protein whose known function is to bind sperm to the zona pellucida and to sulfated carbohydrates, such as dextran sulfate (1, 2). However, recently, our laboratory (unexpectedly) discovered the Sp17 gene in rheumatoid arthritis (RA) synoviocytes obtained from female patients, as compared to osteoarthritis (OA) synoviocytes, using differential display (3). Similarly, a study examining the molecular changes in tumor formation and metastasis demonstrated the Sp17 gene in the metastatic stage, but not the transitional phases, of a multi-stage model of murine squamous cell carcinoma (4). Moreover, Sp17 was found to share peptide structure homology with molecules that regulate gene transcription, and with genes that encode cell junction and signal transduction proteins, suggesting additional functions for Sp17 (5).

The role of Sp17 in highly proliferating tissues (e.g. RA and metastatic neoplasias) is unknown. However, the detection of the Sp17 gene in highly proliferating tissues and the structure of the Sp17 protein, suggest that Sp17 may have an alternative function, possibly in the mediation of signal transduction, protein synthesis and/or unregulated growth (5). Thus, the characterization of Sp17 in normal, as compared to diseased tissues, may reveal important data, which will begin to elucidate potential roles for Sp17 in highly proliferating cells.

1.1 Sp17 sequence

Sp17 was discovered during the development of an immunocontraceptive (5). The Sp17 cDNA was first cloned, sequenced, and characterized from rabbit testis and spermatozoa (1, 2). Similar studies have identified the Sp17 transcript in human, primate and mouse testis tissue (6, 7, 8, 9).

The rabbit Sp17 mRNA was described as a spliced cDNA, 1.3kb and 0.9kb in length (1). Similarly, a human testis cDNA library, screened with a rabbit Sp17 probe, revealed two human Sp17 cDNAs (6). The human Sp17 cDNAs were determined to be 1.6kb and 1.3kb in length and were characterized by alternative 5'UTRs. Although the two human Sp17 cDNAs differ by the 5'UTR region, the coding and 3'UTR regions are identical (Figure 1) (6).

Subsequent genomic sequencing revealed that the Sp17 coding region is made up of five exons and four introns extending over approximately 23 kb (6, Chapter 1). In addition the human Sp17 gene was located on chromosome 11.

1.2 Sp17 protein

The human Sp17 protein is a 151 amino acid polypeptide with a calculated molecular weight of 17,408 Da. Sequence analyses of the Sp17 protein indicate that the C-terminal and N-terminal ends may be involved in the primary, and potential alternative, functions of Sp17 (Figure 2) (5).

At the C-terminal end of the Sp17 protein is a putative calmodulin (CaM) binding site exhibiting similarity to the human growth-associated protein 43 (also known as neuromodulin) and to other CaM binding proteins (e.g. myosin and neurogranin) (10, 11, 12). Generally, CaM is known to regulate Calcium (Ca^{++})-dependent proteins and enzymes, which participate in signaling pathways controlling cell growth and proliferation. However, more recent studies suggest that the Sp17 protein may also function as a CaM binding protein (13).

The N-terminus of the Sp17 protein exhibits sequence similarity to the human cAMP-dependent protein kinase type II regulatory subunit (RII). The Sp17 N-terminal region is thought to be responsible for the dimer formation of RII (14). Recent studies have demonstrated that anchoring proteins bind to the regulatory dimer and sequester it to specific subcellular locations such as centrosomes, the actin cytoskeleton, endoplasmic reticulum, golgi, microtubules, mitochondria and the nuclear matrix (15). Thus, anchoring proteins may enable the compartmentalization of the Sp17 protein. Similarly, studies using anchoring inhibitor peptides suggest that molecules with homology to the R subunit, such as Sp17, have a distinct function in the regulation of sperm motility (16). In addition, the N-terminal region of the Sp17 protein also contains key peptide sequences for a C-type lectin, Ca^{++}-dependent galactose binding domain, which may be involved in cell-cell or/and cell matrix recognition processes (17).

1.3 Sp17 expression

Sp17 is a presumed, testes-specific autoantigenic protein whose known function is to bind sperm to the zona pellucida (1, 2). However, it has been suggested that the Sp17 protein may also function as a calmodulin binding protein (13). In addition, recent studies have detected the Sp17 transcript and the Sp17 protein in normal non-testes and neoplastic tissues (4, 7, 18, 19).

For example, Sp17 was previously shown to be testis specific by northern blot analysis (1, 7). However, the Sp17 gene was detected by differential display in normal sheep mucosa-associated lymphoid tissues, including jejunal Peyer’s patches, non-Peyer’s patch jejunum, mesenteric lymph node and retropharyngeal lymph node (18). Moreover, the detection of the Sp17 gene in non-testis tissues is supported by the identification of Sp17 expressed sequence tag (EST) clones isolated from the lung, kidney, ovary, placenta, uterus and B cell cDNA libraries (7).

Sp17 was also detected in neoplastic tissues. For example, the Sp17 gene was detected by differential display in the metastatic stage, but not the transitional phases of a multi-stage murine model of squamous carcinoma (4). In addition, Sp17 was detected in multiple myeloma cells by northern blot and western blot analyses.

The detection of Sp17 in normal and neoplastic tissues suggests an additional function for the Sp17 protein, possibly in the mediation of signal transduction, protein synthesis and/or unregulated growth (5). In addition, because Sp17 mRNA was

detected in testis and neoplastic tissues, as compared to normal non-testes tissues, the Sp17 protein was implicated as a cancer-testis (CT) antigen (19).

2. Cancer-testis antigens

Gene expression is frequently different in neoplastic cells as compared to normal tissues. In particular, normal testicular transcripts and proteins have been identified in a variety of malignant neoplasias. Recently, some of these proteins have been recognized to comprise a group of tumor-specific antigens called cancer-testis antigens (CT) (20). CTs are characterized by: 1) mRNA expression predominantly in testis, 2) gene activation and mRNA expression in multiple human tumors, 3) existence of multiple gene families, and 4) localization of coding genes to chromosome X (21).

In previous studies, the CT antigen families, MAGE, BAGE and GAGE, were discovered by cloning cytotoxic T lymphocyte-recognized antigens expressed in melanoma cells (22, 23, 24). However, recently, serological analysis of recombinant cDNA expression libraries (SEREX) was used to identify cDNAs encoding the CTs from melanoma (SSX2), esophageal cancer (NY-ESO-1) and renal cancer (SCP1) (25, 26, 27). Similarly, new CTs have been discovered by the SEREX method using a testis cDNA library (28). In addition, CTs have been studied and detected in cancers including those of the brain, breast, liver and lung (29, 30, 31, 32). However, CT expression was not detected in normal non-testis tissues (33).

Sp17 exhibits several hallmarks of CT antigens. In particular, a study of Sp17 in normal tissues revealed that Sp17 mRNA is abundantly expressed in normal testis tissue (1, 7). Similarly, the Sp17 gene, mRNA and the Sp17 protein were detected in the metastatic stage of squamous cell carcinoma and in multiple myeloma cells (4, 19). However, Sp17 has yet to be confirmed as a CT antigen.

The role of CT antigens in neoplastic tissues is not fully understood. However, the selectivity of CT expression in neoplastic versus normal non-testis tissues and the immunogencity of CTs, implicates CTs as an immunotherapeutic target in malignant neoplasms (34). Thus, if Sp17 is a CT antigen, it may be an important immunotherapeutic target in malignant neoplasias.

3. Goals of the investigation

Sp17 has been detected in normal non-testes and in highly proliferating tissues (3, 4, 18, 19). Although the function of Sp17 in these tissues is unknown, altered Sp17 gene expression may contribute to changes in pathologic behavior, including pathways involved in signal transduction, cell growth and death, cell recognition and adhesion, angiogenesis, and host immunity. In addition, Sp17 exhibits sequence similarity to the human cAMP-dependent protein kinase type II alpha regulatory subunit and has a CaM binding site on the Sp17 protein. These characteristics may be important in the transcriptional regulation and function of Sp17 in normal non-testes and highly proliferating tissues (e.g. malignant neoplasias and RA). Thus, a detailed characterization of human Sp17 may provide further insight into the regulation, function and evolution of Sp17.

This study is intended to characterize human Sp17. In particular, this study will explore and differentiate between two Sp17 genes in the human genome, an intron-containing gene (Sp17-1) and an intronless pseudogene (Sp17-2), which may have evolved by the retroposition of the Sp17-1 gene. In addition, to begin to characterize the possible regulation mechanisms of Sp17 mRNA, alternative transcriptional start and multiple polyadenylation sites will be investigated. Furthermore, to examine the tissue distribution of Sp17, the Sp17 mRNA and protein will be examined in normal primate and human tissues and in neoplastic cell lines.

4. Figures

5’ UTR (nucleotides 1-748 represent the 5’UTR for the 1.6kb Sp17 cDNA A)

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  1  cgggcgtgca gacaaaatac atggatgtgg tcaaggagcg aatccgttta gctcgacaga ttgagaaatc
 71  tgagtatcgg aacttccagg cttgcctgca caactcttgg gattgagcag gcagcagctg ccctggagat
141  tgagctggaa gaagacatgt ataagggagg aaaagctgac cagcaagaag aacgtcggag acaaagcaga
211  tgaaggttct gaaggaggag ctgcgccacc tgctgtccag ccactgttta cggagagcca gaaaaccaag
281  tatccactca gtctggcaag ccgccccttg cttgtgtctg ccccaagtaa gagcgagtct gctttgagct
351  gtctctccaa gcagaagaag aagaagacaa agaagccgaa gagccacagc cggaacagcc acagccaagt
421  acaagtgcaa attaactggt caagtgtgtc agtgactgca cattggtttc tgttctctgg ctatttgcaa
491  aacctctccc acccttgagt ttcactccac caccaacccc aggtaaaaaa gtctccctct cttccactca
561  cacccatagc gggagagacc tcatgcagat ttgcattgtt ttggagtaag aattcaatgc agcagcttaa
631  tttttctgta ttgcagtgtt tataggcttc ttgtgtgtta aacttgattt cataaattaa aaacaatggt
701  cagaaaaaaa aaaaaaaccg gaaccggcgg caccagctcg gagagaaa
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5’ UTR (nucleotides 749-1210 represent the 5’UTR for the 1.3kb Sp17 cDNA B)

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749                                                      tc gatgttgtag tgaccttcag
771  taaaagagcg gtttttcata gaggtgccgt tttagactac ctatttaaga ggcacgaaaa acaaatacat
841  ctaataggtt aagtaaaaaa ccatctattt cggacaataa aagttatttt ctacacacgt tggtcttcat
911  tttactcgtt aacagtatca tacatccttc taagcttatc tttttgacgt gaaagtgtag tagtatgtct
981  ccacctggca gctatgtagt taatattttt gtctgttgta atgttatcaa gtaccgaaca ttttcctaat
1051 gaaatagtgg aaaagacaac ctttttctcc atttctattt ggatttttag atcacgtaca taacaaggaa
1121 tcgaataaat aatgaagtgt tttataaaga gtatccgtct tggagggaga ttccagttgg gaggttccat
1191 aggcagttct taccaagaag
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CODING REGION

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1211                     atgtcgattc cattctccaa cacccactac cgaattccac aaggatttgg
1261 gaatcttctt gaagggctga cacgcgagat tctgagagag caaccggaca atataccagc ttttgcagca
1331 gcctattttg agagccttct agagaaaaga gagaaaacca actttgatcc agcagaatgg gggagtaagg
1401 tagaagaccg cttctataac aatcatgcat tcgaggagca agaaccacct gagaaaagtg atcctaaaca
1471 agaagagtct cagatatctg ggaaggagga agagacatca gtcaccatct tagactcttc tgaggaagat
1541 aaggaaaaag aagaggttgc tgctgtcaaa atccaagctg ccttccgggg acacatagcc agagaggagg
1611 caaagaaaat gaaaacaaat agtcttcaaa atgaggaaaa agaggaaaac aagtga
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3’ UTR

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1667                                                             ggac actggtttta
1681 cctccaggaa acatgaaaaa taatccaaat ccatcaacct tcttattaat gtcatttctc cttgaggaag
1751 gaagatttga tgttgtgaaa taacattcgt tactgttgtg aaaatctgtc atgagcattt gtttaataag
1821 cataccattg aaacatgcca cttgaagatt tctctgagat catgagtttg tttacacttg tctcaagcct
1891 atctatagag acccttggat ttagaattat agaactaaag tatctgagat tacagagatc tcagaggtta
1961 tgtgttctaa ctattatcaa atgaataaat cctctctatc acatccccca aaaaaaaaaa aaaaaaaaa
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Figure 1. Nucleotide sequence of the proposed human Sp17 cDNA variants (Accession number Z48570) (4). The human Sp17 cDNAs were determined to be 1.6kb (cDNA A) and 1.3kb (cDNA B). The cDNAs are differentiated by the length of the 5’UTR as indicated. Nucleotides common to the 5’UTR of both mRNAs (1180-1210) are underlined. Although the two cDNAs differ by the length of the 5’ UTR, the coding region and 3’UTR sequences are identical.

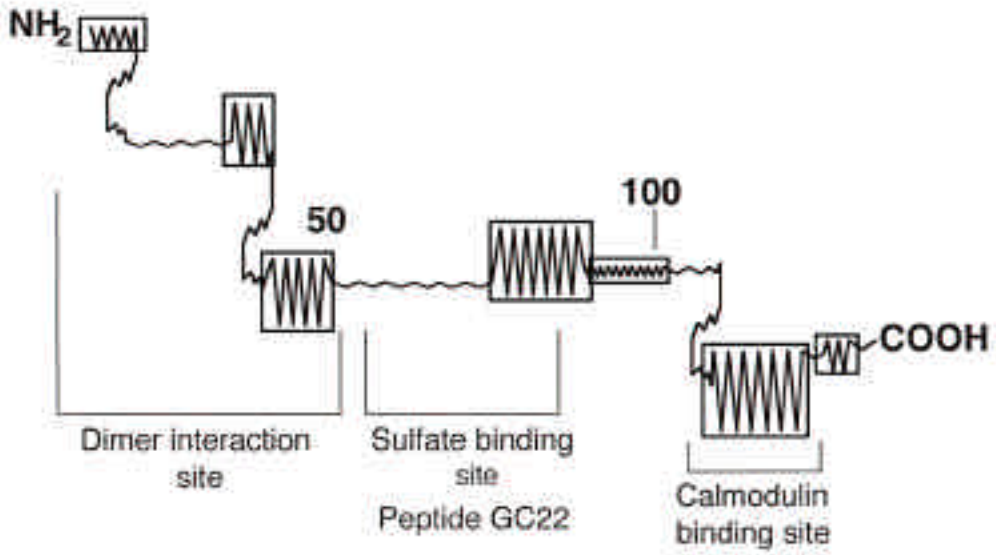

Figure 2. Secondary structure of the human Sp17 protein (1). The helices are depicted as wavy lines and the beta sheets represented by sharp waves. The N-terminal end (NH_2) demonstrates high homology to the human cAMP-dependent protein kinase type II regulatory subunit. The C-terminal end (COOH) contains a putative calmodulin (CaM) binding site.

5. References

1 Richardson, R. T., N. Yamasaki and M. G. O'Rand. 1994. Sequence of a rabbit sperm zona pellucida binding protein and localization during the acrosome reaction. Dev. Biol. 165:688-701.

2 Yamasaki, N., R. T. Richardson and M. G. O'Rand. 1995. Expression of the rabbit sperm protein Sp17 in COS cells and interaction of recombinant Sp17 with the rabbit zona pellucida. Mol. Reprod. Dev. 40:48-55.

3 Takeoka, Y., T. P. Kenny, M. E. Gershwin, H. Yago and D. L. Robbins. Sperm protein 17 gene expression in rheumatoid arthritis synoviocytes. (Submitted)

4 Dong, G., E. Loukinova, C. W. Smith, Z. Chen and C. Van Waes. 1997. Genes differentially expressed with malignant transformation and metastatic tumor progression of murine squamous cell carcinoma. J. Cell. Biochem. Suppl. 28-29:90-100.

5 O'Rand, M. G. and E. E. Widgren. 1994. Identification of sperm antigen targets for immunocontraception: B-cell epitope analysis of Sp17. Reprod. Fertil. Dev. 6:289-296.

6 Lea, I. A., R. T. Richardson, E. E. Widgren and M. G. O'Rand. 1996. Cloning and sequencing of cDNAs encoding the human sperm protein, Sp17. Biochim. Biophys. Acta 1307:263-266.

7 Adoyo, P. A., I. A. Lea, R. T. Richardson, E. E. Widgren and M. G. O'Rand. 1997. Sequence and characterization of the sperm protein Sp17 from the baboon. Mol. Reprod. Dev. 47:66-71.

8 Lea, I.A., B. Kurth and M. G. O'Rand. 1998. Immune response to immunization with sperm antigens in the macaque oviduct. Biol. Reprod. 58:794-800.

9 Kong, M., R. T. Richardson, E. E. Widgren and M. G. O'Rand. 1995. Sequence and localization of the mouse sperm autoantigenic protein, Sp17 [published erratum appears in Biol. Reprod. 1995. 53:1239]. Biol. Reprod. 53:579-590.

10 Chapman, E.R., D. Au, K. A. Alexander, T. A. Nicolson D. R. and Storm. 1991. Characterization of the calmodulin binding domain of neuromodulin. Functional significance of serine 41 and phenylalanine 42. J. Biol. Chem. 266:207-213.

11 Cheney, R.E. and M. S. Mooseker. 1992. Unconventional myosins. Curr. Opin. Cell. Biol. 4:27-35.

12 Rhoads, A.R. and F. Friedberg. 1997. Sequence motifs for calmodulin recognition. Faseb J. 11:331-340.

13 Wen, Y., R. T. Richardson and M. G. O'Rand. 1999. Processing of the sperm protein Sp17 during the acrosome reaction and characterization as a calmodulin binding protein. Dev. Biol. 206:113-122.

14 Taylor, S. S., J. A. Buechler and W. Yonemoto. 1990. cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. Annu. Rev. Biochem. 59:971-1005.

15 Dell'Acqua, M. L. and J. D. Scott. 1997. Protein kinase A anchoring. J. Biol. Chem. 272:12881-12884.

16 Vijayaraghavan, S., S. A. Goueli, M. P. Davey and D. W. Carr. 1997. Protein kinase A-anchoring inhibitor peptides arrest mammalian sperm motility. J. Biol. Chem. 272:4747-4752.

17 Weis, W. I., K. Drickamer and W. A. Hendrickson. 1992. Structure of a C-type mannose-binding protein complexed with an oligosaccharide. Nature 360:127-134.

18 Tatlow, D., R. Brownlie, L. A. Babiuk and P. Greibel. 2000. Differnetial display analysis of gene expression during induction of mucosal immunity. Immunogenetics. 52:73-80.

19 Lim S. H., Z. Wang, M. Chiriva-Internati and Y. Xue. 2001. Sperm protein 17 is a novel cancer-testis antigen in multiple myeloma. Blood. 97:1508-1510.

20 Chen Y. T., L. J. Old. 1999. Cancer-testis antigens: targets for cancer immunotherapy. Cancer J Sci Am. 5:16-7.

21 Chen Y. T., A. O. Gure, S. Tsang, E. Stockert, E. Jager, A. Knuth and L. J. Old. 1998. Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. Proc. Natl. Acad. Sci. 95:6919-6923.

22 Gaugler, B., B. Van den Eynde, P. van der Bruggen, P. Romero, J. J. Gaforio, E. De Plaen, B. Lethé, F. Brasseur and T. Boon. 1994. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. Journal of Experimental Medicine. 179:921-30.

23 Boël, P; C. Wildmann, M. L. Sensi, R. Brasseur, J. C. Renauld, P. Coulie, T. Boon and P. van der Bruggen. 1995. BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity. 2:167-75.

24 Van den Eynde, B., O. Peeters, O. De Backer, B. Gaugler, S. Lucas and T. Boon. 1995. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. Journal of Experimental Medicine. 182:689-98.

25 Türeci, O., Y. T. Chen, U. Sahin, A. O. Güre, C. Zwick, C. Villena, S. Tsang, G. Seitz, L. J. Old and M. Pfreundschuh. 1998. Expression of SSX genes in human tumors. International Journal of Cancer. 77:19-23.

26 Chen, Y. T., M. J. Scanlan, U. Sahin, O. Türeci, A. O. Gure, S. Tsang, B. Williamson, E. Stockert, M. Pfreundschuh and L. J. Old. 1997. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc. Natl. Acad. Sci. USA. 94:1914-8.

27 Türeci, O., U. Sahin, C. Zwick, M. Koslowski, G. Seitz and M. Pfreundschuh. 1998. Identification of a meiosis-specific protein as a member of the class of cancer/testis antigens. Proc. Natl. Acad. Sci. USA. 95:5211-6.

28 Gure, A. O., O. Türeci, U. Sahin, S. Tsang, M. J. Scanlan, E. Jäger, A. Knuth, M. Pfreundschuh, L. J. Old, and Y. T. Chen. 1997. SSX: a multigene family with several members transcribed in normal testis and human cancer. International Journal of Cancer. 72:965-71.

29 Sahin U, M. Koslowski, O. Tureci, T. Eberle, C. Zwick, B. Romeike, J. R. Moringlane, K. Schwechheimer, W. Feiden and M. Pfreundschuh. 2000. Expression of cancer testis genes in human brain tumors. Clin Cancer Res. 6:3916-22.

30 Sahin, U, O. Türeci, Y. T. Chen, G. Seitz, C. Villena-Heinsen, L. J. Old and M. Pfreundschuh. 1998. Expression of multiple cancer/testis (CT) antigens in breast cancer and melanoma: basis for polyvalent CT vaccine strategies [letter] International Journal of Cancer. 78:387-9.

31 Chen C., G. Chen, H. Lee, G. Huang, P. Yang, L. Tsai, D. Chen and J. Sheu. 2001. Expressions of cancer-testis antigens in human hepatocellular carcinomas. Cancer Lett. 164:189-195.

32 Scanlan M. J., N. K. Altorki, A. O. Gure, B. Williamson, A. Jungbluth, Y. T. Chen and L. J. Old. 2000. Expression of cancer-testis antigens in lung cancer: definition of bromodomain testis-specific gene (BRDT) as a new CT gene, CT9. Cancer Lett. 150:155-64.

33 Stockert E., E. Jager, Y. T. Chen, M. J. Scanlan, I. Gout, J. Karbach, M. Arand, A. Knuth and L. J. Old. 1998. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med. 187:1349-54.

34 Chen, Y. T., A. O. Güre, S. Tsang, E. Stockert, E. Jäger, A. Knuth and L. J. Old. 1998. Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. Proc. Natl. Acad. Sci. USA. 95:6919-23.