Published in final edited form as:
Leuk Res. 2008 December ; 32(12): 1889–1894. doi:10.1016/j.leukres.2008.03.036.

Semenogelin I expression in myeloma cells can be upregulated pharmacologically

Yana Zhang[1,2], **Zhiqing Wang**[1,2], **Jian Zhang**[1,2], **Benjamin Farmer**[1,2], and **Seah H Lim**[1,2]

1*Cancer Research Program, Harrington Regional Medical Center*

2*Biotherapy and Stem Cell Transplant Program, Texas Oncology Cancer Center, Amarillo*

Abstract

Semenogelin (SEMG) I is a Cancer-Testis (CT) antigen that we have found to be expressed in myeloma cells. In this study, we set out to determine whether the expression of SEMG I could be upregulated pharmacologically. We found that SEMG I expression in myeloma cells can be upregulated by 5-azacytidine, IL-4 and IL-6. The mechanisms of SEMG I gene upregulation by 5-azacytidine is unclear since there was no correlation between the methylation of the single CpG dinucleotide at position -11 and SEMG I expression. Both IL-4 and IL-6 appeared to enhance SEMG I expression through increasing its promoter function.

Keywords

Semenogelin I; CT antigen; 5-azacytidine; IL-4; IL-6

INTRODUCTION

SEMG I is a major protein of semen coagulum responsible for inhibiting human sperm capacitation (1). It is a non-glycosylated protein in 439 amino acid residues with a molecular weight of 50 kD (2). It plays an important role in sperm clotting and is normally degraded into smaller fragments by prostate-specific antigen. The gene encoding SEMG I has been localized to the long arm of chromosome 20 (3), a region of chromosome 20 that is frequently deleted in myeloproliferative diseases and myelodysplastic syndrome (4). SEMG I shows limited expression in normal tissues except prostate (5) and retinal pigment epithelial cells (6). SEMG II has been previously found to be aberrantly expressed in lung cancer cells and malignant melanoma cells (7). However, SEMG I aberrant expression in tumor cells has only been found in hematologic malignancies, including multiple myeloma (MM) (8), although soluble SEMG I has been detected in the serum of a small proportion of patients with lung cancer (9). The aberrant expression of SEMG I in tumor cells of hematologic malignancies is associated *in vivo* with the generation of high titers IgG directed at SEMG I protein, suggesting the immunogenicity of the protein in the cancer-bearing patients. The combination of being immunogenic in cancer patients and limited expression in normal tissue expression makes SEMG I a potential candidate protein for tumor vaccines.

Correspondence to: Seah H Lim, MD, PhD, FRCP (Lond.), FRCPath., Texas Oncology Cancer Center, 1000 Coulter Drive, Amarillo, TX 79106, Tel. No. 1-806-358 8654, Fax No. 1-806-356 9303, Email: seah.lim@usoncology.com.

One major problem preventing effective clinical tumor immunotherapy in general is the heterogeneity of tumor antigen expression, even within individual specimens. Furthermore, unless the tumor antigen is vital for the survival of the tumor cells, tumor antigens are usually only detected in the tumor cells from a proportion of patients. For SEMG I, the expression frequency in MM was less than 10%. Further study into methods to upregulate SEMG I expression may, therefore, provide vital information relevant to future design of tumor vaccines targeting SEMG I.

We previously showed that DNA methylation regulates the expression of other tumor genes such as *Sp17* (10) and *SPAN-XB* (11,12). Another group also reported that the expression of some tumor antigens that show the predominance of expression in genital tissues is associated with global DNA hypomethylation in the tumor genome (13). In the present study, we have set out to determine whether SEMG I expression could be upregulated pharmacologically and if so, whether or not DNA methylation regulates SEMG I aberrant expression in myeloma cells.

MATERIALS AND METHODS

Materials

Fresh myeloma cells were obtained, after informed consent and approval by the Institution Review Board, from the bone marrow of six patients with a diagnosis of MM. Myeloma cells were enriched by CD138 positive selection (Miltenyi Biotec, Auburn, CA) and used for the experiments. Four tumor cell lines were also used: ARK-B and ARP-1 (gifts from Joshua Epstein, PhD, University of Arkansas for Medical Sciences), RPMI 8226 (gifts from Raymond Comenzo, MD, Memorial Sloan Kettering Cancer Center) and IM 9 (gifts from Dharminder Chanhan, PhD, Dana Farber Cancer Center). Except for RPMI 8226 cells, all the other three cell lines do not express SEMG I, by RT-PCR.

In vitro treatment of myeloma cells with 5-azacytidine and cytokines

Myeloma cells were treated with varying concentrations of 5-azacytidine and a panel of cytokines in RPMI 1640 medium supplemented with 10% FCS. The supplemented medium was replaced daily with fresh culture medium and after 96 hours, the cells were used for experiments. Control cultures were grown under identical conditions but without any pharmacologic agents.

Northern blot analysis

Total RNA (10 μg) was electrophoresed on a 1.2% (w/v) agarose/formaldehyde gel and transferred onto a nitro-cellulose membrane. ^{32}P-labeled probe (derived from a plasmid containing the full length SEMG I cDNA) was hybridized at 60°C overnight; final washes of the membranes were carried out at 60°C with 0.1xSSC in 0.1% (w/v) sodium dodecyl sulphate (SDS) solution.

Immunocytochemical staining

Cytospin specimens were prepared and air-dried and fixed. The fixed cells were treated with 0.4% Triton X solution before the addition of diluted SEMG I murine monoclonal antibodies (in-house, unpublished) for 2 hours. Following washing, the slides were overlaid with a peroxidase-conjugated rabbit anti-mouse secondary antibody for 30 minutes. The slides were washed again in PBS and overlaid with diaminobenzidine reagent. Following washing in PBS, the slides were counterstained in hematoxylin.

Reverse transcription-polymerase chain reaction (RT-PCR) for SEMG I gene segment

First strand cDNA was synthesized from 1 μg of total RNA with random hexamer primers using the GeneAmp RNA PCR Core Kit (Roche Molecular Biochemicals, Indianapolis, IN). The PCR primers were: *5'-CAA AAA GGT GGA TCA AAA GGC-3'* and *5'-TAC TAT GAG CTC ATC TTG GGT-3'*. They amplify a cDNA of 706 bp. PCR was performed with 30 amplification cycles at an annealing temperature of 55 °C. Positive control amplification contained a plasmid with the SEMG I cDNA amplified from human testis RNA by RT-PCR and a negative control contained all the PCR reaction mixture except for substitution of cDNA by water. RNA integrity in each sample was checked by the amplification of a 615 bp β-actin gene segment with primers: *5'-GGC ATC GTG ATG GAC TCC G-3'* and *5'-GCT GGA AGG TGG ACA GCG A-3'* at an annealing temperature of 67 °C. PCR products were visualized on an ethidium bromide agarose gel for DNA band of the expected size. All results were confirmed in 2 independent RT-PCRs.

Sodium bisulfite genomic DNA modification

Genomic DNA was first digested with *EcoRI* and denatured with 0.3 M NaOH for 15 minutes. The denatured DNA was reacted with 3.6 M sodium bisulfite and 1 mM hydroquinone (55 ° C for 14 hours). The DNA was de-salted using a DNA clean up kit (Wizard DNA Clean Up, Promega) and precipitated for PCR. PCR was carried out using optimized oligonucleotide primers that amplify the bisulfite-modified SEMG I promoter: *5'-GTT AGA AGA AAG AAA ATG-3'* and *5'-ATC TTA CTT AAA AAA CCT-3'*. They amplify a gene segment spanning the CpG dinucleotide at position -11 and produce a PCR product of 210 bp. The PCR products were cloned into the TA-cloning system. Recombinant clones were randomly picked from each transfection for nucleotide sequence analysis to determine the proportion of hypomethylated promoter sequence.

Generation of reporter plasmids, cell transfection and analysis of Chloramphenicol Acetyl Transferase (CAT) expression

Full length SEMG I promoter sequence was amplified from genomic DNA derived from normal testis and cloned into the TA cloning system for sequence analysis. PCR primers for the amplification of SEMG I promoter were: *5'-GCG GTA CCC ATG AAG GGA AAC TCA CAT TT-3'* and *5'-GCA GAT CTT GAG AGC TGA GCC GAC CTT GT-3'* and amplified a DNA segment of 348 bp spanning the nucleotides from position −1 to −348 from the transcription start site. Following sequence confirmation, the promoter DNA was subcloned into the pCAT*3-Enhancer vector between *Kpn I* and *Bgl II*.

Transfection was carried out using the FuGENE 6 reagent (Roche Molecular Biochemicals). Briefly, the cells were seeded into a six-well cluster plate. The cultures were transfected with 2 μg of the recombinant plasmids and assayed for CAT activities after 72 hours. FAST CAT Green (deoxyl) Chloramphenicol Acetyltransferase Assay Kit (Molecular Probes, Eugene, OR) was used to detect CAT activity. The transfectants were first lysed and a cytoplasmic extract prepared. The extract was then incubated with the fluorescent deoxylchloramphenicol substrate and acetyl CoA at 37 °C. The reaction was terminated by the addition of ice-cold ethyl acetate. After drying and dissolution in ethyl acetate, the reaction substrate and product were resolved by thin-layer chromatography (TLC) on silica gel plates and eluted with a chloroform:methanol mixture (85:15 v/v). All experiments were carried out in triplicates. Quantitation of the products was carried out using the Bio-Rad Quantity One software (version 4.5.2) (Bio-Rad Laboratories, Hercules, CA). The amounts of the products from the CAT analysis were calculated; % = (product)/(product + substrate) × 100%.

RESULTS

Upregulation of SEMG I gene and protein expression by IL-4 and IL-6

We first determined whether cytokines increase SEMG I expression in SEMG I-positive myeloma cells. A panel of cytokines was tested at varying concentrations on RPMI 8226 cells. The cytokines included IL-2, IL-4, IL-6, IL-7 and GM-CSF. RPMI 8226 cells expressed low basal level of SEMG I before cytokine treatment. Following treatment of the cells with IL-4 and IL-6, SEMG I mRNA levels were upregulated in a dose dependent manner, as shown by Northern blot analysis (Figure 1a). The increase in the mRNA levels was accompanied by corresponding increases in the SEMG I protein, as determined by Western blot analysis on tumor cell lysates (Figure 1b). The other cytokines did not have any effect on SEMG I expression of RPMI 8226 cells.

The ability of these cytokines to induce SEMG I expression in SEMG I-negative myeloma cells was next determined using IM 9 cells. Unlike in RPMI 8226 cells, none of the cytokines, including IL-4 and IL-6, were able to induce SEMG I expression in IM 9 cells. These results suggest that IL-4 and IL-6 only play a secondary role in the regulation of SEMG I expression in myeloma cells. They are only functional if the SEMG I gene has already been switched on.

Upregulation of SEMG I gene and protein expression by 5-azacytidine

We next determined whether or not 5-azacytidine treatment of myeloma cells induces SEMG I expression. Three SEMG I-negative (ARK-B, IM 9 and ARP-1) and one SEMG I-positive (RPMI 8226) myeloma cells were pre-incubated with varying concentrations of 5-azacytidine. The hypomethylating agent was able to not only increase the basal level of SEMG I expression in SEMG I-positive myeloma cells (RPMI 8226) but also induced the expression of SEMG I gene (detected by RT-PCR) in myeloma cells that was SEMG I-negative (ARK-B, IM 9 and ARP-1) (Figure 2). The induction of SEMG I gene expression was associated with a corresponding increase in the SEMG I protein expression, as determined by immunocytochemistry (Figure 2). Additionally, when 5-azacytidine (2 μM) were used to treat fresh CD138-enriched myeloma cells from patients with MM, SEMG I protein was induced in myeloma cells that did not originally express the protein (Figure 3). Since 5-azacytidine is a DNA hypomethylating agent, these results suggest that DNA methylation may be the primary regulatory mechanism for SEMG I expression in myeloma cells.

SEMG I promoter only has one single CpG dinucleotide, located at position -11 of the gene. No other CpG dinucleotide was identified either within or near the SEMG I gene. To determine whether SEMG I gene expression was regulated through SEMG I promoter hypomethylation, bisulfite conversion and nucleotide sequencing was carried out on the genomic DNA from myeloma cells that are either SEMG I-positive (RPMI 8226) or SEMG I-negative (ARP-1, ARK-B and IM 9). We found that the sequences among these tumor cells, whether SEMG I-positive or SEMG I-negative, were all unmethylated at the single CpG dinucleotide at position -11. Bisulfite conversion and sequence analysis was repeated in IM 9 after treatment of the cells with 5-azacytidine. 5-azacytidine treatment of the cells resulted in the expression of SEMG I but did not change the methylation status at this CpG dinucleotide. These results suggest that SEMG I expression in myeloma cells is not regulated through promoter methylation. The mechanisms by which 5-azacytidine upregulate SEMG I expression remains speculative but may be through hypmethylating a SEMG I repressor gene.

IL-4 and IL-6 upregulate SEMG I expression through SEMG I promoter

Having demonstrated the effect of IL-4 and IL-6 on SEMG I expression, we next determined whether these cytokines enhance SEMG I expression through the SEMG I promoter. RPMI 8226 cells transfected with the pCAT*3 Enhancer-SEMG I-promoter vectors were pre-

incubated with these cytokines and then tested for the function of the promoter in inducing chloramphenicol acetyltransferase activities in the cell lysates of the transfectants. Both IL-4 and IL-6 were able to upregulate the SEMG I promoter function in these transfectants (Figure 4). In contrast, IL-2, IL-7 and GM-CSF were not able to do so. Cells transfected with the empty reporter vector did not produce any chloramphenicol acetyltransferase activity. The effects of IL-4 and IL-6 on the function of the SEMG I promoter were dose dependent (Figures 5a and 5b). These results, therefore, support the notion that IL-4 and IL-6 enhance SEMG 1 expression by altering the promoter function of the SEMG I gene.

DISCUSSION

Despite recent advances in the understanding of the molecular events of immune activation, the results of clinical studies on tumor vaccines have been generally disappointing. It has not been possible to reproduce the laboratory successes in the clinic. One of the problems preventing the success is the heterogeneity of tumor antigen expression because most tumor antigens are cellular molecules that are not essential for the neoplastic process or the survival of the tumor cells. Therefore, successful targeting of one particular tumor antigen may be followed by tumor relapse due to the emergence of antigen-negative variant tumor cells. Identification of the pharmacologic agents capable of upregulating the expression of the target genes may, therefore, provide the opportunity to upregulate the expression of the target gene to circumvent antigen deficiency within individual tumor specimen.

SEMG I is one of the predominant proteins of human seminal plasma and it originates from the glandular epithelium of the seminal vesicles. It is a secreted protein that is highly sensitive to the proteolytic degradation by prostate-specific antigen. It was originally thought to be prostate-specific but further investigations found the expression of the protein to be also expressed in kidney, trachea, retinal pigment epithelial cells and skeletal muscles, at much lower levels (5,6). We have previously found SEMG I to be aberrantly expressed by tumor cells from hematologic malignancies (8). The limited normal tissue expression profile, the *in vivo* immunogenicity of the protein in cancer-bearing patients and the aberrant expression of the protein in tumor cells, therefore, make SEMG I is potential target for tumor vaccine. In this study, we set out to determine whether SEMG I could be upregulated using pharmacologic agents.

Using a panel of cytokines, we first demonstrated that SEMG I expression could in fact be upregulated by IL-4 and IL-6. However, these cytokines appeared to provide only a secondary regulatory function since they were only effective in inducing SEMG I in tumor cells already expressing SEMG I. SEMG I expression in myeloma cells that did not express SEMG I was not affected by these cytokines. The effects of these cytokines on SEMG I expression appears to be mediated through the promoter sequence since treatment of transfectant cells with these cytokines resulted in the augmentation of the promoter function in our reporter gene assays.

Supported by our findings that 5-azacytidine-treated myeloma cells express SEMG I, we then investigated the role of promoter methylation in SEMG I expression since genetic anomalies, resulted from increased CpG islands and decrease DNA methylation, occur frequently in malignant diseases and lead to the inactivation of many well-characterized tumor suppressor genes as well as the inactivation of DNA repair genes (14,15). Furthermore, methylation of the CpG dinucleotides is essential for transcriptional regulation (16,17) of certain genes. The ability of 5-azacytidine to induce SEMG I expression supports the role of DNA methylation in the regulation of SEMG I expression. However, we were unable to demonstrate any correlation between the single CpG dinucleotide at position -11 with the SEMG I promoter sequence and SEMG I expression. This is obviously not surprising since it would be highly unusual for one single CpG dinucleotide within the promoter sequence to be responsible for

the regulation of the whole gene. Although 5-azacytidine may upregulate SEMG I expression through a mechanism completely unrelated to its DNA hypomethylating property, it is most likely that the SEMG I gene is constitutively active and its regulation and limited expression within normal tissues is controlled via a repressor gene that is regulated by DNA methylation. Therefore, when cells not expressing SEMG I are treated with a DNA hypomethylating agent, the repressor gene is hypomethylated with the consequence of de-repressing the SEMG I gene.

A previous work showed that SEMG proteins were associated with the cell surface adhesion complexes in small cell lung cancer and that epidermal growth factor induced SEMG protein expression (7). Whether or not the epidermal growth factor induced SEMG expression in small cell lung cancer via the same mechanisms, as we have found for IL-4 and IL-6 in myeloma cells, through the SEMG I promoter remains unclear, although it is unlikely that the epidermal growth factor will have significant effect on myeloma cells that are not expected to express the epidermal growth receptor.

In conclusion, we have demonstrated in the present study that SEMG I expression in myeloma cells can be upregulated pharmacologically using IL-4, IL-6 or 5-azacytidine. Our results suggest the possible use of these agents to increase SEMG I expression for optimal immune targeting of SEMG I for MM. Obviously, it remains to be determined whether or not there is a differential dose response among the different normal tissues in their sensitivity to these agents, in particular, to the hypomethylating effect of 5-azacytidine. Unfortunately, this question could only be answered if these agents are administered to patients and then cells from different normal tissues are tested for their expression of SEMG I. Even so, since the susceptibility of a target cell expressing an antigen to cytotoxic T lymphocyte-mediated lysis depends not only on the gene being expressed and translated but also on factors such as whether or not the protein is processed and presented appropriately in the context of MHC molecules, and whether the number of copies of the MHC-peptide complex present on the surface of the target cells is sufficient to mediate efficient effector-target cell interaction, the mere upregulation of the protein on some normal tissues may not necessarily predict for tissue injury by the cytotoxic T-lymphocytes.

Acknowledgement

Funded by grants from the National Institute of Health/National Cancer Institute (RO1 CA 088434 and RO1 CA 106283).

REFERENCES

1. De Lamirande E, Yoshida K, Yoshiike TM, Iwamoto T, Gagnon C. Semenogelin, the main protein of semen coagulum, inhibits human sperm capacitation by interfering with the superoxide anion generated during this process. J. Androl 2001;22:672–679. [PubMed: 11451365]
2. Lilja H, Abrahamsson PA, Lundwall A. Semenogelin, the predominant protein in human semen. Primary structure and identification of closely related protein in the male accessory sex glands and on the spermatozoa. J. Biol. Chem 1989;264:1894–1900. [PubMed: 2912989]
3. Ulvsback M, Lazure C, Lilja H, Spurr NK, Rao VV, Loffler C, Hansmann I, Lundwall A. Gene structure of semenogelin I and II. The predominant proteins in human semen are encoded by two homologous genes on chromosome 20. J. Biol. Chem 1992;267:18080–18084. [PubMed: 1517240]
4. Asimakopoulos FA, White NJ, Nacheva E, Green AR. Molecular analysis of chromosome 20q deletions associated with myeloproliferative disorders and myelodysplastic syndromes. Blood 1994;84:3086–3094. [PubMed: 7949181]
5. Lundwall A, Bjartell A, Olsson AY, Malm J. Semenogelin I and II, the predominant human seminal plasma proteins, are also expressed in non-genital tissues. Mol. Human Reprod 2002;8:805–810.

6. Boniha VL, Rayborn ME, Shadrach K, Lundwall A, Malm J, Bhattacharya SK, Crabb JW, Hollyfield JG. Characterization of semenogelin proteins in the human retina. Exp. Eye Res 2006;83:120–127. [PubMed: 16545373]
7. Rodriguez RG, Panizo-Santos A, Cashel JA, Krutzsch HC, Merino MJ, Roberts DD. Semenogelins are ectopically expressed in small cell lung carcinoma. Clin. Cancer Res 2001;7:854–860. [PubMed: 11309333]
8. Zhang Y, Wang Z, Liu H, Giles FJ, Lim SH. Pattern of gene expression and immune responses to Semenogelin 1 in chronic hematologic malignancies. J. Immunother 2003;26:461–467. [PubMed: 14595213]
9. Bert A, Virgili A, Errico G, Vespi G, Lago G, Cavazzana A. Expression of seminal vesicle-specific antigen in serum of lung tumor patients. J. Forensic Sci 2005;50:1114–1115. [PubMed: 16225216]
10. Wang Z, Zhang Y, Ramsahoye B, Bowen D, Lim SH. Sp17 gene expression in tumor cells is regulated by promoter methylation. Brit. J. Cancer 2004;91:1597–1603. [PubMed: 15381930]
11. Wang Z, Zhang J, Zhang Y, Lim SH. SPAN-Xb expression in myeloma cells is dependent on promoter hypomethylation and can be upregulated pharmacologically. Int. J. Cancer 2006;118:1436–1444. [PubMed: 16187275]
12. Wang Z, Zhang J, Zhang Y, Srivenugopal KS, Lim SH. SPAN-XB core promoter sequence is regulated in myeloma cells by specific CpG dinucleotides associated with the MeCP2 protein. Int. J. Cancer. 2006 Oct 11;[Epub ahead of print]
13. Sigalotti L, Coral S, Altomonte M, Natali L, Gaudino G, Cacciotti P, Liberner R, Colizzi F, Vianale G, Martini F, Tognon M, Jungbluth A, Cebon J, Maraskovsky, Mutti L, Maio M. Cancer testis antigens expression in mesothelioma: role of DNA methylation and bioimmunotherapeutic implications. Brit. J. Cancer 2002;86:979–982. [PubMed: 11953832]
14. Ng MH, Chung YF, Lo KW, Wickham NW, Lee JC, Huang DP. Frequent hypermethylation of p16 and p15 genes in multiple myeloma. Blood 1997;89:2500–2506. [PubMed: 9116295]
15. Ng MH, Wong IH, Lo KW. DNA methylation changes and multiple myeloma. Leuk. Lymphoma 1999;34:463–472. [PubMed: 10492069]
16. Esteller M. CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. Oncogene 2002;21:5427–5440. [PubMed: 12154405]
17. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature 2004;429:457–463. [PubMed: 15164071]

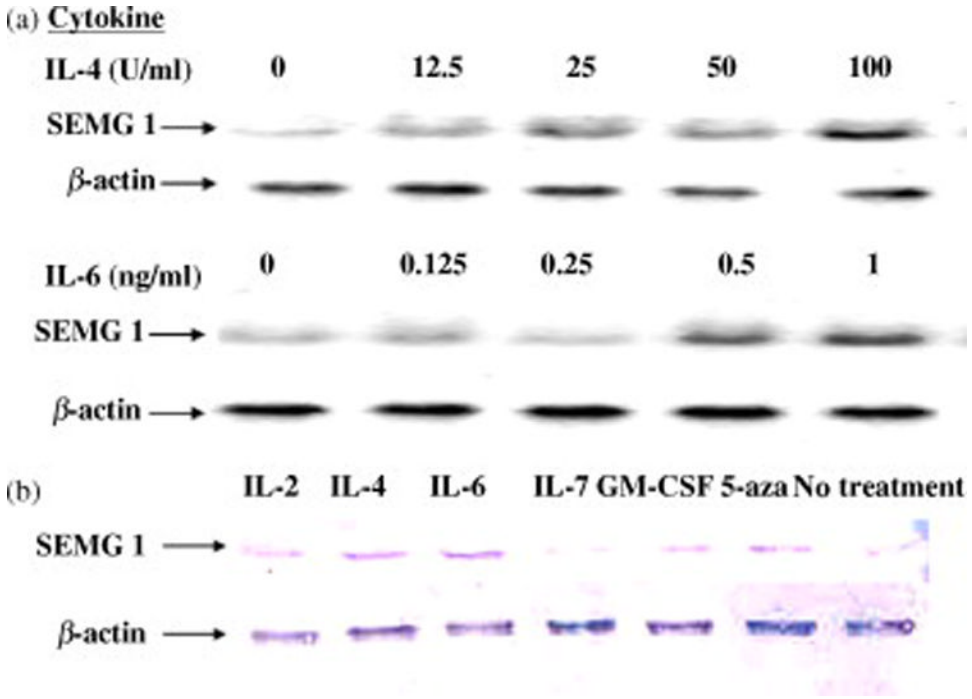

Figure 1.

Treatment of RMPI 8226 cells with IL-4 and IL-6 resulted in the upregulation of SEMG I expression. **(a)**. Northern blot analysis showing dose responses to IL-4 and IL-6 of SEMG I mRNA copy numbers following treatment of the tumor cells with cytokines. **(b)**. Western blot analysis indicating corresponding increases in SEMG I protein following IL-4 and IL-6 treatment of the tumor cells. In contrast, no significant increase in the SEMG I protein levels was observed following treatment with IL-2, IL-7 and GM-CSF. Treatment of the cells with 5-azacytidine further increased the SEMG I protein level.

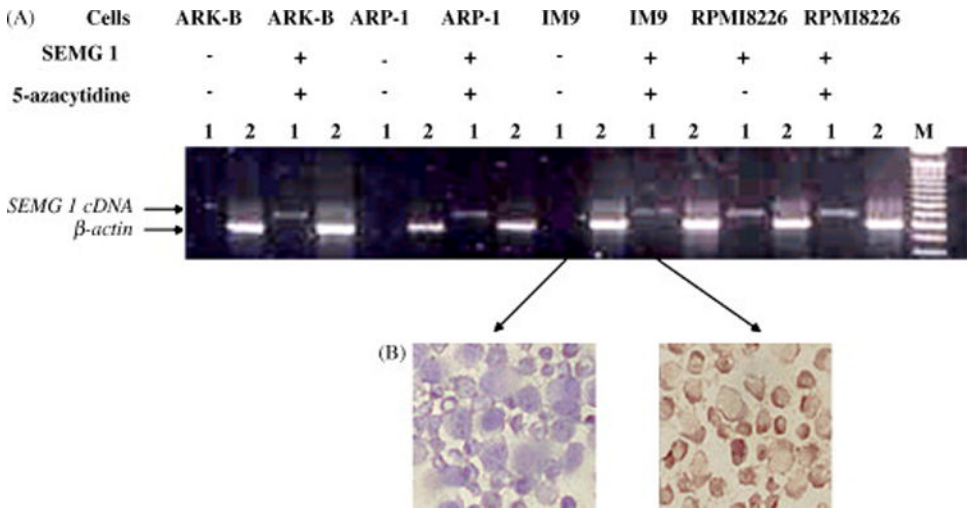

Figure 2.

Effect of treatment with 5-azacytidine on myeloma cell lines. Treatment of myeloma cell lines with 5-azacytidine resulted in: (**A**). Expression of SEMG I mRNA as shown by RT-PCR for the SEMG I gene segment (Lane 1 = RT-PCR for SEMG I gene segment; Lane 2 = RT-PCR for β-actin gene segment; M = molecular marker); (**B**). A corresponding expression of the SEMG I protein in the myeloma cells, as shown by immunocytochemistry.

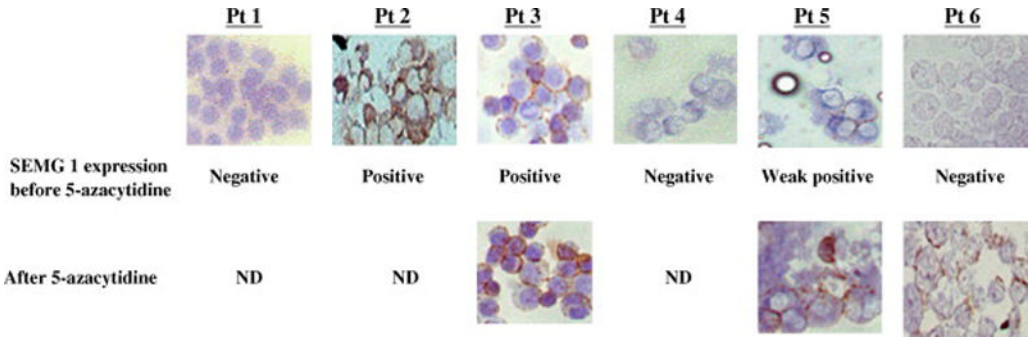

Figure 3.
Treatment of fresh CD138-enriched myeloma cells with 5-azacytidine resulted in the expression of SEMG I protein in the myeloma cells that did not originally express SEMG I, as shown by immunocytochemistry.

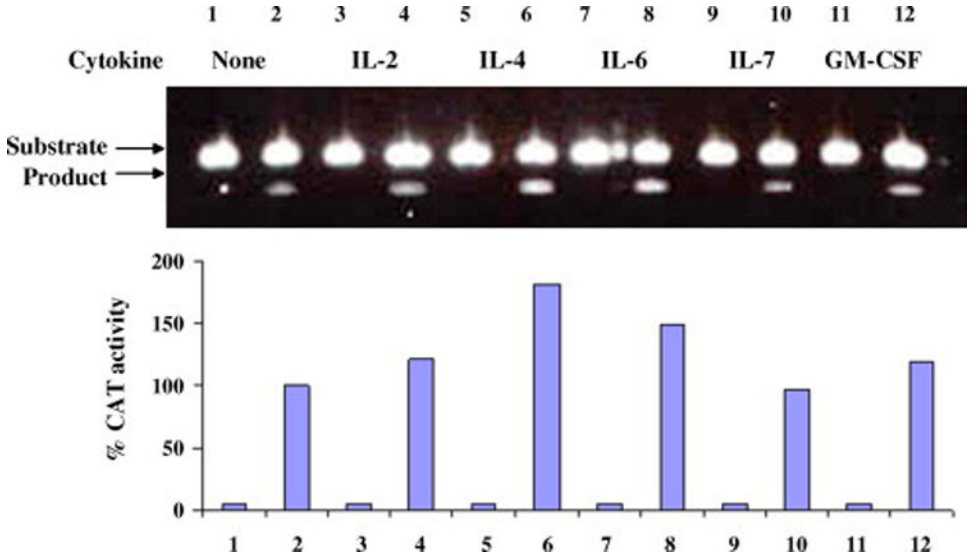

Figure 4.

Effects of various cytokines on the function of SEMG I promoter. Both IL-4 and IL-6 were able to upregulate the function of full length SEMG I promoter significantly. In contrast, IL-2, IL-7 and GM-CSF were unable to do so (Lanes 1, 3, 5, 7, 9, 11 = Cells transfected with empty reporter vector; Lanes 2, 4, 6, 8, 10, 12 = Cells transfected with reporter vectors containing the SEMG 1 promoter sequence.

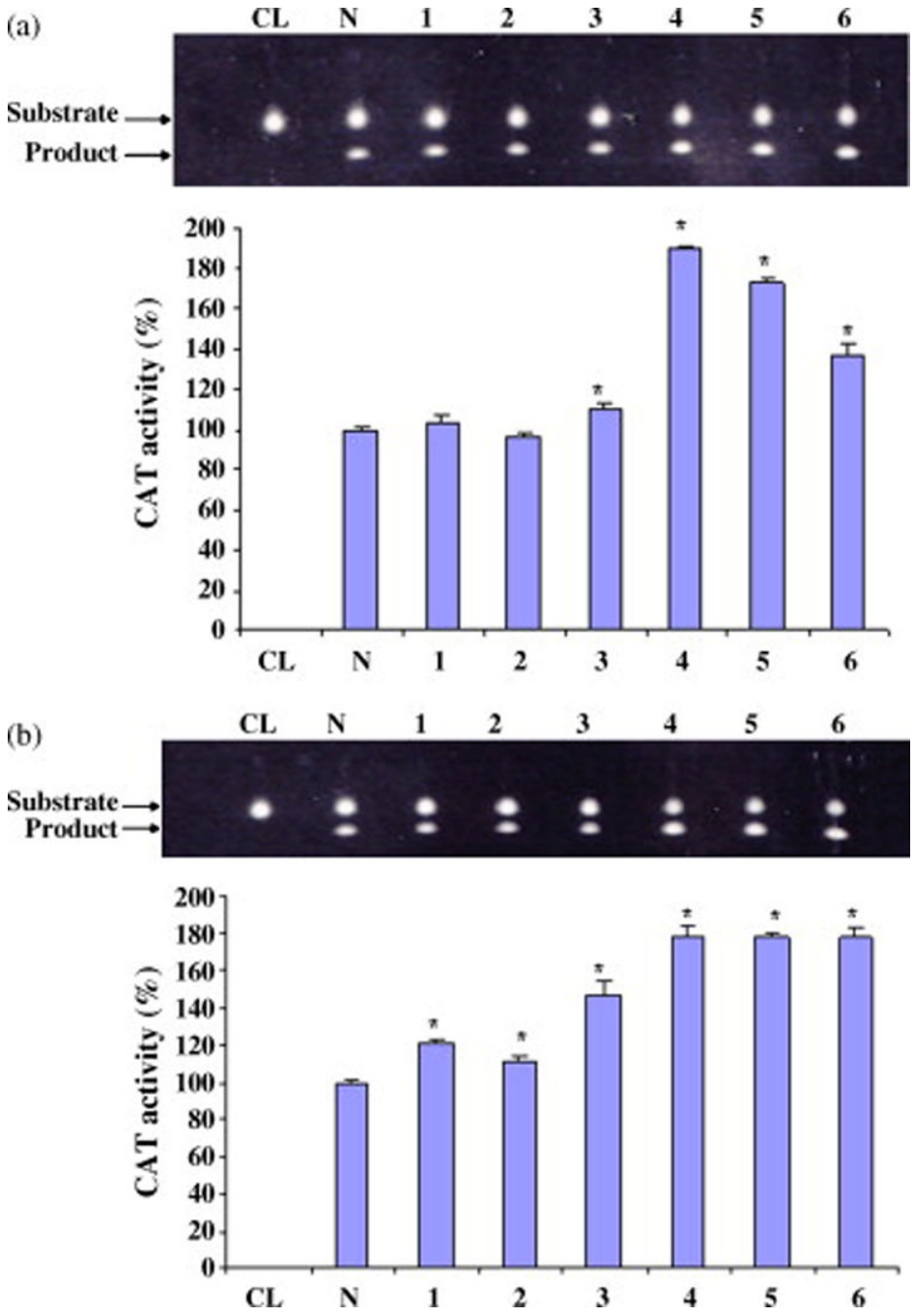

Figure 5.

Dose response effects of IL-4 and IL-6 on SEMG I promoter function. **(a)**. Changes in the effects of varying concentrations of IL-4 on the function of full length SEMG I promoter (CL = empty reporter vector; N = medium only; Lane 1 = 6.25 U/ml; Lane 2 = 12.5 U/ml; Lane 3 = 25 U/ml; Lane 4 = 50 U/ml; Lane 5 = 100 U/ml; Lane 6 = 200 U/ml). (**b**). Changes in the effects of varying concentrations of IL-6 on the function of full length SEMG 1 promoter (CL = empty reporter vector; N = medium only; Lane 1 = 0.0625 ng/ml; Lane 2 = 0.125 ng/ml; Lane 3 = 0.25 ng/ml; Lane 4 = 0.5 ng/ml; Lane 5 = 1 ng/ml; Lane 6 = 2 ng/ml) (* = $p < 0.05$).