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Expression of an alternate splice form of Bmi-1 in multiple myeloma

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Mentor – Dr. James Tung
Nevada Cancer Institute

Expression of an alternate splice form of Bmi-1 in multiple myeloma

The concept of "tumor stem cells" has garnered much attention in the last few years. Tumor stem cells are believed to exist among a heterogeneous group of cells that constitute a tumor. These tumor stem cells often express genes that are important for stem cell function, cell division, and maintenance of pluripotent state in stem cells. Stem cell or stem cell maintenance genes such as SALL 4 and Bmi-1 are often seen in these cancer cells and contribute to self-renewing divisions and cancer cell survival. In particular, high expression of Bmi-1 (B lymphoma mouse Moloney leukemia virus insertion region), a member of the polycomb family of transcription factors, is often associated with poor prognosis in cancers.

Our laboratory has shown the existence of an alternatively spliced Bmi-1 RNA and protein in multiple myeloma cells. The purpose of this research project is to understand the effect of an alternate splice form of Bmi-1 protein on cell cycle and apoptosis in multiple myeloma cells. To understand the effect of this alternate Bmi-1 protein, I first compared the growth rate of different myeloma cell lines and correlated that with the expression of the wild-type Bmi-1 and the alternatively spliced Bmi-1 form. I performed time course experiments and counted the cell numbers in each cell line at various time points. My results show that the myeloma cell lines, which highly express the alternate form of Bmi-1, grew faster than the myeloma cell lines, which mostly express the wild-type form of Bmi-1. Currently I am performing RT-PCR and western blot analysis to confirm the existence of the alternatively spliced Bmi-1 protein. I plan to isolate and sequence the alternatively spliced form of Bmi-1. I also plan to determine the effect of knocking-down Bmi-1 expression on cell cycle and apoptosis by incorporating inducible shRNA viral constructs targeted against Bmi-1 RNA in myeloma cells.

Expression of an alternate form of Bmi-1 in multiple myeloma

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Abstract

Tumor stem cells are believed to exist among a heterogeneous group of cells that constitute a tumor. Stem cell or stem cell maintenance genes such as SALL 4 and Bmi-1 are often seen in these cancer cells and contribute to cell divisions and cancer cell survival. In particular, high expression of Bmi-1, a member of the polycomb family of transcription factors, is often associated with poor prognosis in cancers.

Our laboratory has shown the existence of an alternatively spliced Bmi-1 RNA and protein in multiple myeloma cells. The purpose of this research project is to understand the effect of an alternate splice form of Bmi-1 protein on cell cycle and apoptosis in multiple myeloma cells.

We show here that an alternate-spliced form of Bmi-1 is found in a myeloma cell line, RPMI. The alternate-spliced form can also be observed on the protein level in western blot analysis. We have purified and sequenced the alternate-spliced Bmi-1 RT-PCR product and determined alternate splicing regions. Furthermore, we show that the RPMI (myeloma) and NB4 (AML)[1] cell lines have higher growth rate compared to other myeloma cell lines. Since both RPMI and NB4 both express predominantly the alternate-spliced Bmi-1 form, the growth rate results suggest that the expression of the alternate form may confer growth advantage in cancer cells.

Background

Bmi-1 (B lymphoma mouse Moloney Leukemia virus insertion region) is one of the genes regulated by SALL4 and is a member of the polycomb family of transcription factors. The over-expression of Bmi-1 can be found in a variety of cancers such as breast cancer, glioma, nasopharyn-geal cancer, and mantle cell lymphomas[2,3].

While Bmi-1 is shown to be involved in the pathogenesis of several cancers, very little is known whether Bmi-1 plays a role in the pathogenesis and progression of multiple myeloma (MM), a cancer of plasmacytoid cells characterized by elevated monoclonal antibodies and bone destruction. The pathogenesis of multiple myeloma requires both dysregulation of apoptosis and cell cycle.

We hypothesize that Bmi-1 is expressed and acts as a key regulator of cell growth and apoptosis in multiple myeloma cells. Our preliminary data supported this hypothesis. Using flow cytometry and western blot analysis, We demonstrated that Bmi-1 is detected in five myeloma and one myeloid leukemia cell lines.

While Bmi-1 expression is found in these cell lines, our laboratory also found that RPMI and NB4 cell lines expressed an alternate form of Bmi-1 protein. This alternate form is smaller in size and predominates in these two cell lines. Based on these results, we hypothesize that while Bmi-1 expression may increase cell division, the expression of this alternate Bmi-1 form produces a dominant negative effect and further induces the myeloma cells into cell replication and division.

Literature Cited

1. Yang, J., L. Chai, F. Liu, L.M. Fink, P. Lin, L.E. Silberstein, H.M. Amin, D.C. Ward, and Y. Ma. 2007. Bmi-1 is a target gene for SALL4 in hematopoietic and leukemic cells. *Proc Natl Acad Sci USA.* 104:10494-10499.
2. Mihic-Probst, D., A. Kuster, S. Kilgus, B. Bode-Lesniewska, B. Ingold-Heppneer, C. Leung, M. Storz, B. Seifert, S. Marino, P. Schraml, R. Dummer, and H. Moch. 2007. Consistent expression of the stem cell renewal factor Bmi-1 in primary and metastatic melanoma. *Int J Cancer.* 121:1764-1770
3. Jacobs, J.J., K. Kieboom, S. Marino, R.A. DePinho, and M. van Lohuizen. 1999. The oncogene and Polycomb-group gene Bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature.* 397:164-168

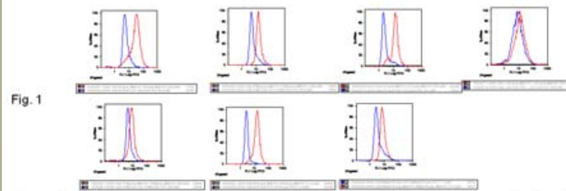
Objectives

1. **Confirm, isolate and sequence the alternate Bmi-1 protein in myeloma cells.**
2. **Determine whether the alternate Bmi-1 protein correlates with increased cell division.**

Results

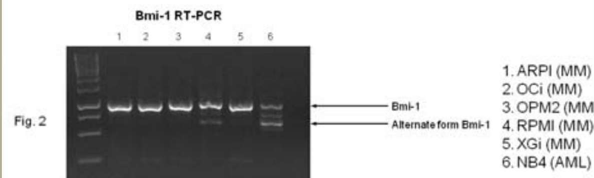
Presence of an alternate form of Bmi-1

Fig. 1



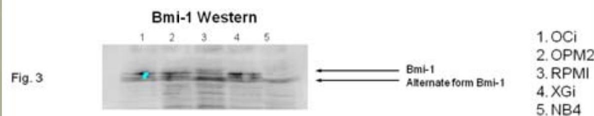
·**Figure 1 Bmi-1 is expressed in several multiple myeloma cell lines.** Bmi-1 expression in multiple myeloma cell lines is detected by flow cytometry. Bmi-1 staining is shown in red. Background staining from FITC-conjugated secondary antibody is shown in blue.

Fig. 2



·**Figure 2. DNA analysis of five different myeloma cell lines and an acute myeloid leukemia cell line (NB4) using RT-PCR (reverse transcription-polymerase chain reaction).** An alternate form of Bmi-1 is expressed in both RPMI and NB4 cells. The alternate form of Bmi-1 is roughly 300bp shorter than the expected size.

Fig. 3



·**Figure 3 Western blot analysis also shows the expression of an alternate form of the Bmi-1 protein.** Both RPMI (lane 3) and NB4 (lane 5) express predominantly the alternate splice form of Bmi-1 (lower band).

Nucleotide sequence of alternative form Bmi-1

Fig. 4

```
tgtgtgctttgtggagggtacttcattgatgccacaaccataatagaatgtctacattccttctgtaaaacgtgtattgttcgttacctggagaccagc
aagtattgtcctatttgtgatgtccaagttcacaagaccagaccactactgaatataagattgatgtcatgtatgaggaggaacctttaaaggattatt
atacactaatggatattgcctacatttatacctggagaaggaatggtccacttccattgaaatacagagttcgacctacttgtaaaagaatgaagat
cagtcaccagagagatggactgacaaatgctggagaactggaaagtgactctgggagtgacaaggccaacagcccagcaggagtactatga
atggaaccagcaacagccccagcggtaaccaccaatcttcttttgccaatagacctcgaaaatcatcagtaaatgggtcatcagcaacttctctg
ggttaggagmggtaaccaccaatcttcttttgccaatagacctcgaaaatcatcagtaaatgggtcatcagcaacttcttctggttaggaaa
```

·**Figure 4 Nucleotide sequence of the alternative form of Bmi-1.** To get the sequence, the desired band was cut out from the gel and purified. It was then re-amplified using the same PCR primers that were used initially, and the final product was sequenced.

Fig. 5

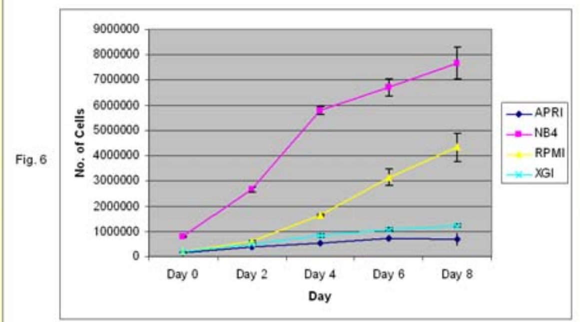
```
Query  1     TGTGTGCTTTGTGGAGGGTACTTCATTGATGCCACAACCATAATAGAATGTCTACATTCC  60
Sbjct  557   TGTGTGCTTTGTGGAGGGTACTTCATTGATGCCACAACCATAATAGAATGTCTACATTCC  616

Query  61    TTCTGTAAAACGTGTATTGTTCGTTACCTGGAGACCAGCAAGTATTGTCCTATTTGTGAT  120
Sbjct  617   TTCTGTAAAACGTGTATTGTTCGTTACCTGGAGACCAGCAAGTATTGTCCTATTTGTGAT  676

Query  121   GTCCAAGTTCACAAGACCAGACCACTACTGAATATAAG  158
Sbjct  677   GTCCAAGTTCACAAGACCAGACCACTACTGAATATAAG  714
```

```
Query  157   AGATTGATGTCATGTATGAGGAGGAACCTTTAAAGGATTATTATACACTAATGGATATTG  216
Sbjct  1074  AGATTGATGTCATGTATGAGGAGGAACCTTTAAAGGATTATTATACACTAATGGATATTG  1133

Query  217   CCTACATTTATACCTGGAGAAGGAATGGTCCACTTCCATTGAAATACAGAGTTCGACCTA  276
Sbjct  1134  CCTACATTTATACCTGGAGAAGGAATGGTCCACTTCCATTGAAATACAGAGTTCGACCTA  1193

Query  277   CTTGTAAAAGAATGAAGATCAGTCACCAGAGAGATGGACTGACAAATGCTGGAGAACTGG  336
Sbjct  1194  CTTGTAAAAGAATGAAGATCAGTCACCAGAGAGATGGACTGACAAATGCTGGAGAACTGG  1253

Query  337   AAAGTGACTCTGGGAGTGACAAGGCCAACAGCCCAGCAGGAG  376
Sbjct  1254  AAAGTGACTCTGGGAGTGACAAGGCCAACAGCCCAGCAGGAG  1293
```

·**Figure 5 Determination of alternate spliced Bmi-1 region.** The splice junction (bp714-1074) was determined using nucleotide BLAST to published Bmi-1 sequence.

Growth rate for multiple myeloma cell lines

Fig. 6



	APRI Cell Count	NB4 Cell Count	RPMI Cell Count	XGI Cell Count
Day 0	157849	787618	191802	174614
Day 2	391979	2651714	610992	510772
Day 4	518295	5795535	1633515	829476
Day 6	735045	6704954	3142014	1083868
Day 8	684209	7680768	4330468	1239331

·**Figure 6 RPMI and NB4 have higher growth rates.** Time course experiments were performed to compare the growth rate of different cell lines. From there a comparison of the expression of wild-type Bmi-1 and the alternate Bmi-1 form could be made. The cell lines which highly express the alternate Bmi-1 form (NB4, RPMI) grew much faster than those cell lines which express the wild-type Bmi-1 (XGI, APRI).

Future Plans

Determine the effect of knocking-down Bmi-1 expression on cell cycle and apoptosis by incorporating inducible shRNA viral constructs targeted against either Bmi-1 wild type and/or variant mRNA in myeloma cells. Then:

1. **Determine the effect of altering Bmi-1 expression on cell cycle.**
2. **Determine the effect of altering Bmi-1 expression on apoptosis.**