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

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Repository Citation
Austin, Adam; Veys, Kristine; Wong, Debbie; and Tung, James, "Expression of an alternate splice form of Bmi-1 in multiple myeloma" (2009). Undergraduate Research Opportunities Program (UROP). 3.
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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 SALL4 and Bmi-1 are often implicated 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 splice form of Bmi-1 is found in a myeloma cell line. RT-PCR and flow cytometry were used to detect the alternate splice form. The results suggest that the expression of the alternate form may confer growth advantage in cancer cells.

Background

Bmi-1 (B lymphoma mouse c-moi ony 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, nasopharyngeal cancer, and mantle cell lymphoma. 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 plasma 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 myeloma and one myeloid leukemia cell lines.

While Bmi-1 expression is found in these cell lines, our laboratory also found that RPMI and NIBA cell lines express 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 viability, the expression of an alternate Bmi-1 form produces a dominant negative effect and further reduces the myeloma cells into cell repopulation and division.

Literature Cited


Figure 1: Expression of Bmi-1 in several multiple myeloma cell lines. Bmi-1 expression was determined by flow cytometry. Bmi-1 staining is shown in red. Background staining is shown in blue.

Figure 2: Western blot analysis of the alternate form of Bmi-1 protein. Both RPMI-1640 and NIBA-1 (lane 3) express predominantly the alternate splice form of Bmi-1 (low band).

Figure 3: Western blot analysis of the alternate form of Bmi-1 protein. Both RPMI-1640 and NIBA-1 (lane 3) express predominantly the alternate splice form of Bmi-1 (low band).

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.