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The Bmi-1 polycomb group gene in skin cancer: regulation of function by (–)–epigallocatechin-3-gallate

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The PcG genes encode a family of evolutionarily conserved regulators that were discovered in *Drosophila* as repressors of *Homeotic* gene expression. Homeotic genes are required to establish body segmentation patterns during development. In mammalian systems, PcG proteins regulate genes involved in development and differentiation, via epigenetic (e.g., chromatin modification) mechanisms. They are intimately involved as silencers of gene expression during cell lineage determination, and they play an important role in promoting cell survival. Proteins encoded by PcG genes comprise two protein complexes that act coordinately to regulate gene expression – the Bmi-1 complex and the Eed complex. The Bmi-1 complex includes Bmi-1, Mel-18, Mph1/Rae28, M33, Scmh1, Ring1A, and Ring1B, while the Eed complex includes Eed, EzH1, and EzH2. The first step in gene silencing is mediated by the Eed complex. Eed-containing complexes modify chromatin by recruiting histone deacetylase, which leads to local chromatin deacetylation. The Eed complex also catalyzes the methylation of Lys₂₇ of histone H3. In the second step, the Bmi-1 complex binds to the methylated Lys₂₇ of histone H3 and then catalyzes the ubiquitinylation of histone H2A. This cooperation between the Eed and Bmi-1 complexes leads to silencing of

gene expression. The Bmi-1 complex appears to remain anchored to the chromatin after these events are completed. The Bmi-1 PcG protein has a particularly important role in gene silencing due to its ability to enhance the rate and extent of histone ubiquitinylation. This is accomplished through activation of Ring 1B, a ubiquitin ligase (and PcG protein) present in the Bmi-1 complex.^{1,2}

Bmi-1 also functions in normal adult tissues. For example, aging Bmi-1^{-/-} mice progressively lose stem cells in the leukemic, neuronal, and cerebellar granule cell lineages³⁻⁵ and Bmi-1-deficient fibroblasts display enhanced senescence and slow proliferation rates.⁶ In contrast, Bmi-1 overexpression enhances cell proliferation.⁴ Bmi-1 is also overexpressed in some human cancers, including colorectal cancer,⁷ and human non-small-cell lung cancer.⁸ In addition, Bmi-1 overexpression immortalizes human mammary epithelial cells via a mechanism that involves increased levels of human telomerase reverse transcriptase and increased telomerase activity.⁹ Thus, Bmi-1 functions as a pro-survival regulator.

BMI-1 IN NORMAL HUMAN EPIDERMIS

The epidermis is a multilayered tissue in which basal cells divide to give rise to suprabasal differentiated cells.¹⁰ The differentiated cells are living but unable to divide. Ultimately, these cells terminally differentiate to form dead cells that are lost from the epidermal surface. We believe that epigenetic regulation is important in the control of this process, so we initiated studies to examine the role of the PcG genes, particularly Bmi-1.¹¹ We propose that Bmi-1 plays an important role in maintaining proliferation of basal keratinocytes and in maintaining the viability of suprabasal differentiating keratinocytes.

As assessed by immunoblot and by reverse transcriptase polymerase chain reaction (RT-PCR), Bmi-1 is present in cultured human epidermal keratinocytes. Moreover, immunolocalization studies, using antibody against Bmi-1, indicate that Bmi-1 is present in the nucleus of these cells. We further confirmed that Bmi-1 is expressed in epidermis by RT-PCR by using total RNA prepared from isolated human foreskin epidermis. Immunolocalization studies reveal that Bmi-1 is present in the basal and suprabasal epidermal (spinous and granular) layers, but that Bmi-1 is not present in the dermis.^{11,12} To examine the functional role of Bmi-1 in normal human keratinocytes, we overexpressed Bmi-1 using an adenoviral vector and then monitored changes in cell number and morphology. These studies reveal that Bmi-1 increases cell number without altering cell morphology. We also showed that Bmi-1 protects cells against challenge with stress agents. In these experiments, keratinocytes were infected with tAd5-EV or tAd5-hBmi-1. At 24 h post-infection the cells were treated with okadaic acid, a pro-differentiation/pro-apoptotic agent, and at 48 h post-infection the cells were examined. This study showed that Bmi-1 expression protects the cells from the cell death-promoting effects of okadaic acid and preserves normal cell morphology, lending credence to the hypothesis that Bmi-1 is a pro-survival protein.¹¹

One possibility is that Bmi-1 enhances cell survival by altering the level of cell cycle regulatory proteins.^{3,7,13,14} Indeed, our findings indicate that Bmi-1 expression increases cdk2 and cdk4, as well as the level of cyclin D1, suggesting that Bmi-1 influences cell proliferation. In addition to regulating proliferation, Bmi-1 may also influence cell survival by suppressing apoptosis. In keratinocytes, apoptosis is associated with loss of mitochondrial membrane potential, release of cytochrome *c* and caspase activation,¹⁵⁻¹⁷ and cleavage of poly(ADP-ribose) polymerase (PARP).¹⁷ Our studies show that overexpression of Bmi-1 in keratinocytes reverses the okadaic acid-dependent increase in apoptosis. Thus,

these findings represent the first evidence that Bmi-1 and the Bmi-1 PcG complex can inhibit apoptosis.¹¹

DIETARY AGENTS, SKIN CANCER, AND BMI-1 EXPRESSION

We were interested in whether Bmi-1 may mediate or antagonize the ability of chemopreventive agents to reduce skin cancer cell survival. Skin cancer is among the most common forms of cancer and is caused by environmentally mediated damage to DNA by agents such as ultraviolet light.¹⁸ An important strategy is to inhibit cell division and/or reduce the survival of cancer cells, or to make the cells more sensitive to co-therapy. This goal can be achieved by reducing the level or activity of cell survival factors such as the PcG proteins. Because they promote cell proliferation and survival and operate via an epigenetic mechanism, PcG proteins have great potential as anti-cancer therapeutic targets. Despite the fact that these genes are overexpressed in some cancer cell types, and are potential anti-cancer targets, the role of diet-derived chemopreventive agents in modulating PcG function has not been adequately investigated.

We recently reported that Bmi-1 levels are markedly elevated in immortalized and transformed epidermis-derived cell lines and in epidermal squamous cell carcinoma.¹¹ To examine the functional role of Bmi-1 in these cancer cells and to assess whether chemopreventive agents modulate Bmi-1 function, we used SCC-13 cells – a transformed cell line derived from squamous cell carcinoma. We have studied several chemopreventive agents, including the active ingredient from green tea, (–)-epigallocatechin-3-gallate (EGCG). EGCG inhibits skin carcinogenesis in mice,¹⁹ and also inhibits proliferation of a range of skin cancer cell lines.²⁰ One important observation is that treatment with EGCG results in reduced Bmi-1 levels. Keratinocytes were treated for 24 h with EGCG prior to harvest and assayed for Bmi-1 level by immunoblot (unpublished). This treatment results in a marked suppression of Bmi-1 level. In addition, the residual Bmi-1 migrates more slowly, which is indicative of increased phosphorylation (unpublished). Bmi-1 phosphorylation is associated with loss of Bmi-1 from the nucleus and a reduction in activity of the Bmi-1 PcG complex.²¹

We next monitored for an impact of Bmi-1 on cell morphology and cell number. SCC-13 cells were incubated with empty (tAd5-EV) or Bmi-1 encoding (tAd5-hBmi-1) adenovirus for 24 h followed by treatment for 48 h with 40 μ M EGCG. The cell number doubled over the time course of the experiment for the empty virus (tAd5-EV) treated group. However, in the presence of the Bmi-1 expressing adenovirus, cell number increased fourfold (unpublished data). Thus, as anticipated from our studies using normal keratinocytes,¹¹ Bmi-1 increases cell number. Treatment with EGCG markedly inhibits proliferation of the empty vector group, but does not substantially reduce cell number in the Bmi-1 overexpressing group. This finding indicates that Bmi-1 can antagonize the EGCG-dependent reduction in cell number.

To understand the underlying mechanism responsible for the Bmi-1 antagonism of EGCG action, we monitored the impact of Bmi-1 and EGCG on cdk4 expression (cell cycle) and the level of intact PARP (apoptosis). EGCG treatment results in a reduction in cdk4 level, which is associated with reduced cell number. When Bmi-1 is present, however, the EGCG-dependent suppression is largely reversed (unpublished data). In addition, EGCG treatment results in increased PARP cleavage, indicating that EGCG treatment increases apoptosis, but when Bmi-1 is present, this response is largely reversed (unpublished data). Thus, Bmi-1 effectively inhibits the anti-proliferation and pro-apoptotic actions of EGCG.

SUMMARY

The results of our studies support several conclusions. First, the Bmi-1 PcG gene product is expressed at high levels in skin tumors and in cultured squamous cell carcinoma cells, indicating that Bmi-1 may play a role in enhancing skin tumor cell survival. Second, Bmi-1 is present in a wide range of cells in tumors.¹¹ Thus, although Bmi-1 has been thought to be restricted to maintaining stem cell survival, the ubiquitous distribution of Bmi-1 in skin tumor cells suggests a broader role in skin cancer. Third, our studies suggest that Bmi-1 may be a downstream mediator of EGCG action. This idea is supported by the finding that treatment with EGCG reduces Bmi-1 level and increases Bmi-1 phosphorylation (unpublished). Increased Bmi-1 phosphorylation is associated with reduced Bmi-1 activity.²¹ We therefore propose that EGCG may reduce cancer cell survival by altering epigenetic control of gene expression, via its ability to reduce Bmi-1 function. Fourth, our studies suggest that Bmi-1 controls the balance between skin cancer survival and death by modulating the balance between cell apoptosis and proliferation (summarized in Fig. 1), and that this is achieved via regulation of cyclin-dependent kinase function and control of the apoptosis cascades.

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Figure 1. Chemopreventive agent action is mediated by the Bmi-1 epigenetic regulator

Bmi-1 is present at elevated levels in skin tumor cells, where it functions to enhance cell survival by increasing cell proliferation and suppressing apoptosis (mediated via effects on both cell cycle and apoptotic regulators). Treatment with the chemopreventive agent EGCG causes a reduction in the Bmi-1 level. This negatively impacts cell number by reducing the tendency of the cells to proliferate and by enhancing their tendency to undergo apoptosis. This model proposes that the Bmi-1 epigenetic regulator mediates some of the chemopreventive effects of EGCG.