Guest Editorial

Mechanisms of Regression

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[See related article pp. 89 - 97]

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In this issue, Bayer-Garner et al.¹ report on the immunopathology of regression in benign lichenoid keratosis, keratoacanthoma (KA), and halo nevus. They suggest that cytotoxic T-cells may be the common denominator of regression in these tumors. Other studies of halo nevi have shown abundant CD8+ T-cells and FXIIIa-positive histiocytes during regression, suggesting that the immune response is involved in tumor regression.^{2,3} Halo congenital nevi undergoing spontaneous regression also demonstrate infiltrating T-cells, with CD8+ cells outnumbering CD4+ cells. Natural killer cells are not numerous, but IgM antibodies against nevus cells and melanoma cells are noted.⁴ Humoral immunity may play a role in regression, or may simply represent an epiphenomenon. Cell-mediated immunity is consistently observed.

These observations prompt the basic question: why do tumors regress? Spontaneous tumor regression occurs in approximately one in every 140,000 cases of cancer.⁵ The mechanisms involved in tumor regression are complex and interrelated. Understanding these mechanisms may shed light on why some tumors regress completely, while others, such as melanoma, show evidence of regression in the primary tumor simultaneous with the occurrence of distant metastasis.

As demonstrated by Bayer-Garner and others, cytotoxic immune responses appear to play a role in the regression of some tumors. Interleukin (IL)-18 stimulates T and natural killer cell activity, is associated with interferon (IFN)- γ production, and can induce antitumor immune responses.⁶ Tumor-associated antigens that may be targets for CD8+ T cells include viral antigens, melanocyte differentiation antigens, and cancer-testis antigens. Expression of the cancer-testis antigen, NY-ESO-1, has been shown to induce both humoral and cellular immune responses, and is associated with a high rate of regression.⁷ The observation that autoimmune manifestations may occur concomitantly with spontaneous tumor regression also suggests that regression may be immune-mediated.⁸

In melanoma, Mart-1 antigen loss in metastases from patients whose primary melanomas have regressed has led to speculation that tumor regression can be associated with immune surveillance.⁹ Loss of surveillance could account for tumor spread. However, inflammation does not correlate well with regression in melanoma, and clonally expanded T-cells in the tumor do not necessarily exert an effective antitumor response.¹⁰ Clearly, other mechanisms are important in the regression of melanoma.

Regression of melanoma *in situ* may take the form of lichenoid regression, mimicking benign lichenoid keratosis histologically.¹¹ This dense infiltrate appears quite different from the sparse lymphoid infiltrate and fibrosis commonly seen in regression associated with distant metastasis. While partial regression in cutaneous malignant melanoma is often associated with a poor prognosis, regression of KA is associated with an excellent prognosis. The different mechanisms of regression may be related to the prognosis of the tumor.

In KA, regression may relate to terminal differentiation, a phenomenon whereby the tumor simply keratinizes itself to death.¹² Terminally differentiated cells are no longer capable of cell division, and are therefore mortal. Terminal differentiation in squamous epithelial cells is manifested by keratinization, and can be monitored by measuring the expression of cytokeratin 10, a marker for terminal keratinization.

The phenomenon of terminal differentiation is well described in other tumors, and often occurs concurrently with inflammation or apoptosis. In acute promyelocytic leukemia, treatment with all-trans-retinoic acid and chemotherapy can result in both terminal differentiation and apoptosis.¹³ Bruceantin can also induce both terminal differentiation and apoptosis in hematopoietic malignancies.¹⁴ Expression of epidermal growth factor receptor (EGFR) in human squamous cell carcinomas is often associated with poor prognosis. Monoclonal anti-EGFR antibodies and inhibitors of EGFR-associated tyrosine kinase induce both terminal differentiation and apoptosis.¹⁵ Retinoids, such as 9-cis-retinoic acid, can produce both apoptosis and differentiation in neuroblastoma cells. The apoptosis occurs after treatment, upon subsequent withdrawal of 9-cis-retinoic acid.¹⁶ Sodium phenylacetate with tamoxifen induces cell differentiation, apoptosis, and an antiangiogenic effect, and results in regression of breast carcinoma cell lines.¹⁷

KAs demonstrate an initial period of rapid growth followed by terminal differentiation and involution. This pattern of growth and involution can be produced in other squamous cell lines. Human papillomavirus-immortalized and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) transformed oral keratinocytes demonstrate a similar initial growth phase followed by terminal differentiation and regression. An inflammatory response is present in the final stages of the tumor.¹⁸

Spontaneous regression of KAs does not occur until the proliferative phase has run its course, but earlier regression can be induced by imiquimod.¹⁹ As imiquimod is a potent stimulator of interferon release, is all KA regression related to cytokine responses?

Compared with squamous cell carcinoma, KAs show an increase in intercellular adhesion molecule-1 expression and

activated (IL-2-receptor positive) CD4+ T-lymphocytes.²⁰ However, spontaneous regression in KAs is associated with a significant increase in IL-10. Spontaneously regressing tumors also show no differences in expression of tumor necrosis factor- α , IL-2, IL-8, IL-13, IFN- γ , or transforming growth factor- β compared to squamous cell carcinoma. These results suggest that although immune stimulation can result in resolution of KAs, terminal differentiation with spontaneous resolution often occurs in an immunosuppressive environment.²¹

Clearly, cell populations may die different deaths. Immunological mechanisms can trigger apoptosis resulting in tumor regression.²² Cells may also follow their normal course of differentiation leading to terminal maturation and mortality. In the setting of chronic myelogenous leukemia (CML), busulphan can induce a pronounced decrease of the white blood cell count and restoration of normal peripheral smear morphology. These effects are not preceded by marrow hypoplasia but rather are related to terminal differentiation (leukemic cells differentiating into granulocyte-like cells).²³ Whereas differentiation therapy in skin cancers involves inducing production of cross-linked protein envelopes resulting in keratinization and cell death, non-physiologic cross-linking may also result in cell death. Methyl-2,5-dihydroxycinnamate, a kinase inhibitor, induces non-physiologic cross-linking in a variety of mouse and human squamous skin cancer cell lines. It can also produce protein cross-linking in a fibroblast and a melanoma cell line.²⁴

Spontaneous regression of solitary cutaneous mastocytomas has been shown to be related to apoptosis.²⁵ Programmed cell death is also involved in spontaneous regression and differentiation of neuroblastomas and may be related to expression of a variety of cell death-related proteases.²⁶ Spontaneous regression of some neuroblastomas is associated with a form of Ras-mediated programmed cell death that is caspase cascade-independent (non-apoptotic).²⁷ Monoclonal antibodies to human EGFR induce terminal differentiation (keratinization and expression of involucrin and cytokeratin 10) in xenograft tumors that overexpress EGFR. They also induce a host mononuclear cell infiltrate, but do not induce apoptosis.²⁸ These data suggest that apoptosis is often associated with tumor regression but is not required for regression.

Just as apoptosis is not required for regression of all tumors, apoptosis alone may not be sufficient to cause regression. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) and DNA ladder studies demonstrate a high apoptotic rate in Merkel cell carcinoma despite the aggressive behavior of this tumor and its lack of regression.²⁹ Apoptosis is an important mechanism of regression but is neither necessary for nor alone sufficient for regression to occur. Other mechanisms are important.

Angiogenesis is critical for the development of many tumors, and antiangiogenic factors are promising agents in the treatment of cancer. The humanized monoclonal antibody bevacizumab exerts an effect on vascular endothelial growth factor and improves survival in metastatic colorectal cancer, suggesting that antiangiogenic therapy can have a meaningful clinical effect.³⁰ Ornithine decarboxylase (ODC) overexpression is associated with the formation of spontaneous skin carcinomas in some transgenic mice. ODC stimulates dermal vascularization. Treatment with α -difluoromethylornithine, an inhibitor of ODC, causes a decrease in blood vessel count and regression of the tumors.³¹ Other promising antiangiogenic agents include thalidomide, IFN- α , and matrix metalloproteinases. Pharmacologic antiangiogenic factors can produce tumor regression, but the role of endogenous antiangiogenic factors in spontaneous regression remains a fertile area for research.

Tumor regression may be an immune-mediated event, may reflect a process of terminal differentiation, or may be related to vascular compromise. Genomic instability is another mechanism for tumor regression. Telomerase is associated with cellular immortality and tumorigenesis. Inhibition of telomerase may result in genomic crisis and tumor regression.³² In neuroblastoma, high levels of telomerase activity correlate with poor outcome, whereas telomere shortening correlates with tumor regression.³³ Spontaneously regressing cutaneous melanoma in Sinclair swine demonstrates telomerase activity during the growth phase but no detectable telomerase activity in regressing melanoma. Regressing tumors demonstrate a reduction of telomeric repeats and abnormal telomeric multicentric and ring configurations. Breakage-fusion-bridge-cycles result in the loss of these configurations, DNA fragmentation, and cell death.³⁴

Telomere shortening can result in regression, but is also associated with disease progression in some cancers, such as CML. In patients with CML, telomere repeat array reduction is related to the time course of disease acceleration and may identify patients at high risk of disease transformation.³⁵ Genomic crisis occurs when telomeres are exhausted. Crisis results in cell death, but out of this crisis deadly cell lines may emerge. Comparative genomic hybridization has demonstrated chromosomal aberrations in melanoma, suggesting that telomeric crisis may be involved in both regression and progression of melanoma.^{36,37} This may explain why the primary melanoma can regress at the same time that the tumor metastasizes.

If genomic crisis is related to regression in melanomas, while terminal differentiation accounts for regression of KAs, this could explain the differences in prognosis between melanoma with regression and regressing KA. Regression through genomic crisis may be a riskier venture than regression through terminal differentiation.

A complex interplay of mechanisms is involved in tumor growth and tumor regression. Neoplastic transformation is related to the expression of oncogenes, the production of growth factors, and inactivation of tumor suppressor genes. Regression, on the other hand, may be mediated by the immune response, apoptosis, antiangiogenesis, terminal differentiation, or genomic crisis resulting from telomere exhaustion. Better understanding of these mechanisms may give us a better ability to predict tumor behavior and to effect cures.

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