

Implications of Epstein–Barr Virus (EBV)-Induced Carcinogenesis on Cutaneous Inflammation and Carcinogenesis: Evidence of Recurring Patterns of Angiogenesis and Signal Transduction

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In a recent issue of the Proceedings of the National Academy of Sciences, we found that Burkitt's lymphoma caused by Epstein–Barr Virus (EBV) used differing signaling pathways than sporadic Burkitt's lymphoma (Cerimele *et al*, 2004). EBV infection was associated with high levels of reactive oxygen and mitogen-activated protein (MAP) kinase signaling, whereas EBV-negative Burkitt's lymphoma had very low levels of either reactive oxygen and MAP kinase signaling. Significantly, EBV infection was associated with hypermethylation of the p16ink4a tumor suppressor gene, whereas EBV negative lymphomas were associated with mutation of the p53 tumor suppressor gene. Finally, we show that when EBV-positive lymphoma cells are treated with a potent inhibitor of reactive oxygen, ebselen, nuclear factor κ B (NF κ B) is inhibited, but MAP kinase is activated. What do these findings have to do with cutaneous carcinogenesis and inflammation?

The three most common cancers of the skin are basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. In BCC and SCC, mutations of p53 are extremely common, whereas in melanoma, loss of p16ink4a is very common (Ziegler *et al*, 1993; Hussussian *et al*, 1994). The most important carcinogen in terms of the skin is ultraviolet light, with UVB capable of causing direct DNA damage as well as reactive oxygen generation, whereas UVA is less damaging to DNA, but causes generation of reactive oxygen (Tsao and Sober, 1998). We have previously shown that reactive oxygen-generating carcinogens cause hypermethylation of p16ink4a, and our current paper shows that EBV infection, as well as specific EBV genes, such as latent membrane protein-1 (LMP-1) generate reactive oxygen (Govindarajan *et al*, 2002). It thus stands to reason that UV-induced reactive oxygen may play a major role in the development of melanoma.

Why is melanoma so much more aggressive than the average BCC or SCC? The answer may lie in the choice of signaling pathways. We have previously hypothesized that p16-deficient tumors can use a variety of signaling pathways, including MAP kinase, PI3 kinase/akt, and reactive oxygen signaling (Arbiser, 2004). P53-deficient tumors are more limited in their ability to use differing signaling pathways, i.e., p53-deficient tumors are often highly sensitive to reactive oxygen-generating agents such as X-irradiation, whereas p16-deficient tumors are more resistant (Arbiser, 2004). Our data also demonstrate another important feature

of tumors. They are capable of switching signaling mechanisms in the face of strong inhibition of a signaling pathway (Fig 1). The tumor cell uses a balance of signaling mechanisms, and inhibition of one pathway leads to rapid adaptation to another pathway. Some of the strongest evidence of this comes from clinical practice. Velcade is a potent inhibitor of NF κ B signaling that is currently first-line therapy for multiple myeloma, a tumor known to have inactivation of p16ink4a as a common event (Barlogie *et al*, 2004; Jagannath *et al*, 2004). Although velcade has impressive activity in myeloma, resistance occurs commonly, and may be because of compensatory upregulation of MAP kinase signaling. Velcade has been given to patients with metastatic melanoma, with less than impressive results. Although velcade may reduce NF κ B activation in metastatic melanoma, MAP kinase activation likely compensates, given the known role of MAP kinase in melanoma development (Cohen, 2002; Govindarajan, 2003). Similarly, BAY 43-9006, a tyrosine kinase inhibitor directed against B-raf, is not curative of melanoma even though many melanomas have mutant B-raf. Likely, effective antagonism of B-raf occurs *in vivo*, but the tumor responds with NF κ B activation.

Similarly, EBV infection is associated with the development of systemic lupus erythematosus (SLE). In a recent study, patients with SLE were found to have antibodies that cross-react between Epstein–Barr Nuclear Antigen and Ro autoantigen (McClain *et al*, 2005). What do EBV and SLE have in common? Both are known to be associated with overproduction of interleukin-10. In our study of Burkitt's lymphoma, we show that neutralizing antibodies against interleukin-10 block production of reactive oxygen. It is likely that EBV infection of a genetically susceptible individual results in aberrant production of interleukin-10, resulting in B cell proliferation and autoantibody production. Inhibitors of reactive oxygen, such as ebselen described in our study, may be beneficial in antagonizing interleukin-10-induced pathology in both EBV infection and SLE (Liu *et al*, 2005).

Are these discoveries all bad news? The failure of single agent-targeted therapies are disappointing, yet predictable. Yet, this does not spell doom for these novel agents. Now that we know that p16ink4-deficient tumors, which comprise the most aggressive of human tumors, can switch signaling pathways, we can anticipate patterns of resistance. These findings make the argument for concurrent or

Regulation of VEGF Transcription by Alternate Signaling Pathways

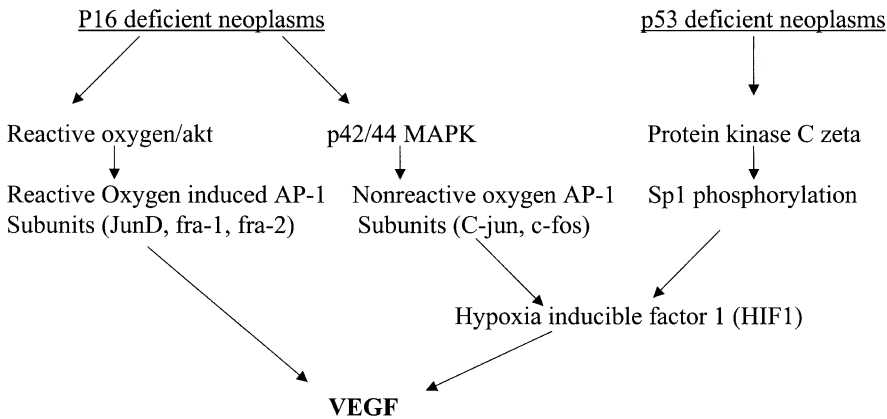


Figure 1

Alternating signaling pathways used by tumor cells. P16-deficient neoplasms are capable of signaling through the P42/44 ERK MAP kinase, akt, and reactive oxygen/nuclear factor κ B (NF κ B) pathways. Blockade of reactive oxygen/NF κ B leads to activation of p42/44 ERK MAP kinase. Blockade of p42/44 ERK may lead to compensatory upregulation of reactive oxygen/NF κ B and akt. VEGF transcription can be controlled by a number of AP-1 subunits, some of which preferentially operate under conditions of high reactive oxygen (JunD, fra-1, fra-2) or low reactive oxygen (c-jun, c-fos). Induction of VEGF under conditions of low reactive oxygen often occurs through the hypoxia inducible factor (HIF1) pathway, whereas high reactive oxygen favors HIF-independent pathways.

sequential combination therapies, i.e. Raf/MAP kinase inhibition with NF κ B inhibition. We have discovered multiple ways of inhibiting NF κ B activation, including compounds familiar to the dermatologist. These include velcade, epicatechin gallate, honokiol, curcumin, and other drugs (Arbiser *et al*, 1998; Ahmad *et al*, 2000; Aggarwal *et al*, 2004). In addition, we have developed multiple ways of inhibiting tyrosine kinases, and these drugs include many in clinical use, such as glivec, iressa, herceptin, and BAY 43-9006. Now is the time to study the optimal combinations of these drugs in the treatment of highly malignant disorders, such as melanoma.

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