What are the therapeutic implications of increased collagen expression in melanoma cells treated with vemurafenib?

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“...the pleotropic effects of collagen on cells within the extracellular matrix has led to the suggestion that the collagen functions as a ‘double-edged sword’ in both enhancing and inhibiting melanoma progression.”

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In a recent article published in *Matrix Biology*, M Jenkins, W Croteau, D Mullins and I described the effect of the mutant BRAF^{V600E} inhibitor, vemurafenib/PLX4032, on collagen synthesis in melanoma cells [1]. We found that this small molecule inhibitor, which blocks the sequential MAPK, RAS, RAF, MEK, ERK signaling pathway [2–4], substantially increased the synthesis of type I collagen in several human and murine melanoma cell lines. Importantly, we also found that the MEK inhibitor, U0126, which targets the wild-type MAPK pathway [2–4], also increased collagen production, indicating that this effect is not limited to cells harboring mutant BRAF.

While the majority of our studies were carried out *in vitro*, we confirmed the increased collagen deposition with *in vivo* studies where nude mice with intradermal human xenografts were fed chow containing the drug. Thus, the possibility exists that blocking this mutant MAPK signaling

pathway in BRAF^{V600E} melanomas could have therapeutic implications for patients treated with these inhibitors. But what are these implications? Is increased collagen deposition a ‘good’ or ‘bad’ outcome? Does it correlate with the resistance to vemurafenib, which usually develops within a year of treatment [3,4]?

There may not be a ‘correct’ answer to this question. Type I collagen is the major protein in our bodies, comprising as much as 30% of total protein [5,6]. Further, it is increasingly recognized that as a primary component of the extracellular matrix, collagen is far from an inert scaffold [5,7]. Instead, it is a lively contributor to cellular functions, interacting with normal stromal fibroblasts, cells of the immune system, and tumor cells via integrin signaling and mechanical forces. Some of these interactions result in the induction of matrix metalloproteinases (MMPs), which then, in turn, begin to destroy the matrix, perhaps undoing effects of matrix

KEYWORDS

- BRAF^{V600E} • BRAF^{WT} • carcinoma-activated fibroblasts • fibroblasts
- MAPK signaling • MEK inhibitors
- TGF-β • tumor microenvironment
- vemurafenib/PLX4032

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deposition on both tumor and stromal cells in the tumor microenvironment (TME).

Our results were somewhat surprising, since most effects of blocking the MAPK pathway describe a reduction in the expression of genes [8]. Many of these genes are associated with inflammation, invasion and tumor progression and thus, a reduction in their expression would appear to be a clear therapeutic benefit. Only a few genes were increased, and two of these represent predicted transcripts, while the third is *CITED-2*, a transcriptional coregulator that may suppress the collagenases *MMP-1* and *MMP-13* by acting through the TGF- β pathway. Its upregulation may, therefore, facilitate matrix deposition by reducing expression of these matrix-degrading MMPs.

In trying to decipher the mechanism(s) by which the MAPK inhibitors increased the synthesis of type I collagen, we investigated TGF- β , a major inducer of collagen synthesis [9,10]. However, we were unable to define a clear role for TGF- β , since blocking TGF- β signaling with a specific small molecule inhibitor only partially blocked the increase in collagen expression, suggesting that additional mechanism(s) are involved. Indeed, we examined whether there might be cross-talk between TGF- β signaling and other MAPK pathways, such as pERK, pJNK and pp38 [7,9–10]. Interestingly, we found that exogenous TGF- β and PLX4032 increased pp-38 and that this increase was blocked with a pp-38 inhibitor [1], implicating co-operativity between these pathways in mediating some of the increases in collagen [9,10].

However, other mechanisms must be involved, they may not directly affect transcription. The regulation of collagen gene expression and matrix deposition is complex, involving not only TGF- β signaling, but also signaling via integrins [5] and the tyrosine kinase, discoidin domain receptor [11] and increased stability of collagen mRNA [12].

It is also essential to recognize the contribution of stromal cells in laying down collagen in the TME. Stromal fibroblasts adjacent to the tumor can be activated by soluble factors secreted by the tumor cells to become carcinoma-activated fibroblasts [13,14]. The small molecule inhibitor, such as U0126, blocks MEK in BRAF^{WT} in normal fibroblasts as well as in tumor cells [2–4]. Since both normal fibroblasts and carcinoma-activated fibroblasts are potent sources of collagen, if they increase collagen deposition in response to a

BRAF^{WT} MEK inhibitor, this provides another avenue of increased collagen deposition.

Whether this increase is therapeutically beneficial or detrimental remains unknown. One study has shown that preventing collagen degradation prevented melanoma metastasis [15], implying that degradation of collagen is a necessary step in melanoma progression. Conversely, other studies have shown that type I collagen enhances angiogenesis [6,16], implying that collagen deposition enhances tumor progression. Indeed, the pleiotropic effects of collagen on cells within the extracellular matrix has led to the suggestion that the collagen functions as a ‘double-edged sword’ in both enhancing and inhibiting melanoma progression [5].

The concept of the ‘double-edged sword’ suggests that both scenarios may be correct. As tumorigenesis proceeds, the role of collagen in modulating tumor behavior may change. Initially, collagen may serve as a constraint by preventing tumor growth and migration. However, as growth continues, there are mechanical forces exerted by the tumor that influence these physical constraints and the collagen matrix becomes more forgiving, allowing some migration of tumor cells, perhaps with the collagen fibers serving as ‘tracks’ for cell migration [5–7]. In addition, cytokines and growth factors produced by the tumor cells and/or by the adjacent stromal cells may increase production of MMPs, which degrade collagen and other matrix components, thereby, loosening the hold of the collagen matrix on the tumor to facilitate tumor invasion [5,13–14]. It is important to emphasize that these activities represent an ongoing dynamic situation rather than a static environment. Most likely, there are times during tumorigenesis when the collagen serves a protective function for the host against tumor growth, while at other times, the tumor is the favored component of the system.

Another important variable are cells of the immune system, which are recruited to the tumor microenvironment where their behavior may be altered [5]. Both intact collagen fibers and their degraded products are potent chemoattractant for cells of the innate immune response (macrophages) and adaptive immunity (T cells and B cells). Type I collagen has been associated with reduced cytotoxicity of macrophages against tumor cells. Furthermore, by integrin signaling, collagen may exert immunosuppressive effects by activating certain receptors on T cells, which

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suppress an antitumor response. Thus, high levels of collagen may curb antitumor immunity.

Studies have described the ability of pharmacologic agents directed at blocking collagen synthesis, with the goal of achieving therapeutic benefit. The antifibrotic effects of Losartan were mediated by downregulating TGF- β 1, and resulted in improved delivery of nanotherapeutics in several models of cancer [17]. In another study, the plant alkaloid, halofuginone reduced the establishment and progression of melanoma bone metastases [18]. The ‘take-home message’ from these studies is that collagen deposition increases tumor progression, suggesting that blocking either the wild-type or mutant RAS–RAF–MEK–ERK pathway, with the concomitant increase in collagen production, represents an unwanted and potentially serious off-target effect.

Nonetheless, there are several cautionary notes. First, although we demonstrated that the increase in collagen was seen in several human and murine melanomas, it is essential to point out that melanomas are extremely heterogeneous tumors [19]. Consequently, the actual levels of collagen production may vary considerably from one melanoma to another, and perhaps, even within subpopulations of a particular melanoma. Second, the presence or absence of the BRAF^{V600E} mutation within the melanoma may influence both the inherent level of collagen gene expression and the response of the tumor to MAPK inhibitors. For example, BRAF^{V600E} is associated with increased expression of the cytokine and IL-8, and the increase is blocked with vemurafenib [13]. However, IL-8 suppresses collagen expression in normal fibroblasts [20], and inhibiting its production relieves this suppression, with the potential of increasing collagen expression. Thus, an inverse link between IL-8 suppression and increased production of collagen is possible. Third, the prominent role of stromal cells in collagen deposition needs to be stressed. These cells are

the major players in collagen synthesis, and there is potential of further stimulating collagen gene expression by factors, including TGF- β , secreted by the melanoma cells and/or by blocking MEK.

In the final analysis, understanding the therapeutic implications of increasing collagen synthesis in melanoma progression is a complex process, which may depend on several variables. These include the molecular characteristics of the melanoma cells, themselves and their secreted proteins, as well as their effects on the fibroblasts in the tumor microenvironment. This conclusion reflects the growing recognition of the critical role of stromal fibroblasts within the TME and how it may influence patient outcome. In the absence of a simple ‘yes’ or ‘no’ concerning the therapeutic implication of increased collagen induced by blocking the RAS–RAF–MEK–ERK pathway in melanomas, further basic investigations into the mechanisms behind this increase are needed, along with patient-based outcomes, particularly in clinical trials where combinatorial treatments with vemurafenib and MEK inhibitors are used to delay the onset of resistance to blocking the MAPK pathway [3,4].

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