Concluding remarks

Given the scarcity of effective treatment options available for patients with advanced melanoma, the dawn of nanoparticle delivery systems could not come at a better time. Future studies in the field should focus on potential combinations of siRNA- and chemotherapeutic/ pharmacological inhibitor-containing nanoparticles because both formulations have been shown to increase delivery and effectivenesss. In addition, although syngeneic mouse models such as B16F10 are highly important in the development of cancer therapies, it would be advantageous to extend these studies into models more relevant to human melanoma progression. Specifically, the recent conditional B-RAF^{V600E}/PTEN-deficient metastatic melanoma mouse is an ideal disease model for preclinical studies of these nanoparticles (Dankort *et al.*, 2009). Additional studies with human melanoma xenograft mice, which allow for representation of a wide range of melanoma genetic backgrounds and staging, would also be of value.

In conclusion, increasing evidence from the past several years has revealed nanoparticles to have the potential to improve the efficacy of current treatments and/or to open doors to strategies such as siRNA targeting, which until now have been plagued with problems. The work of Chen *et al*. (2010) extends this area of research and demonstrates that targeting melanoma tumor cells with c-*Myc* siRNA packaged within the new DSAA nanoparticle, alone or in combination with chemotherapy, can inhibit melanoma tumor growth in several systems, offering hope for the development of potent and efficacious treatment strategies for advanced melanoma.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Oxygenation State as a Driver of Myofibroblast Differentiation and Wound Contraction: Hypoxia Impairs Wound Closure

Chandan K. Sen[1] and Sashwati Roy[1]

Myofibroblasts are ubiquitous in the human body and may form from the differentiation of fibroblasts, epithelial cells, endothelial cells, and mononuclear cells, among others. Their clinical significance could be substantial, depending on biomedical context. Myofibroblasts help contract open skin wounds, but they could also be key drivers of fibrosis across numerous tissue systems and support tumor invasiveness. Understanding the molecular events underlying myofibroblast formation is significant for many human diseases. In this issue, Modarressi *et al.* address the significance of wound tissue hypoxia in impairing wound contraction by compromising myofibroblast formation. They present compelling evidence indicating tissue hypoxia conflicts with wound closure. We are reminded that correcting wound tissue hypoxia is critical for the tissue's response to other therapeutic interventions.

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In 1977, Packer reported that human diploid fibroblasts grown at 10% O_2 live longer than cells grown at the routine 20% O_2 (Packer and Fuehr, 1977). The field of cellular senescence was in its infancy, with the concept of the "Hayflick limit" reported in 1961. In 2003, Roy *et al.* (2003a) reported that growth arrest of fibroblasts caused by 20% O_2 was reversible, consistent with the current study (Modarressi *et al.*, 2010), and it was therefore concluded that exposure of cells to hyperoxic insult causes differentiation, but not senescence. Although it is standard practice to culture cells at an ambient O_2 concentration of 20% (i.e.,

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room air balanced with 5% CO_2), which corresponds to a pO_2 of approximately 140 mm Hg at sea level, cells within the human body are exposed to much lower concentrations, ranging from about 14% (100 mm Hg) in pulmonary alveoli to 3–5% (35 mm Hg) in the heart and skin (Roy *et al.*, 2003a; Sen *et al.*, 2006). Thus, it is important to recognize that standard cell culture under conditions of 20% O_2 represents exposure of cells to potentially hyperoxic insult, particularly for primary cells that have been freshly isolated from organs and adjusted to lower pO_2 as their physiological normoxic status (Roy *et al.*, 2003a; Sen *et al.*, 2006). For cell lines cultured at 20% O_2 over a long period of time, it is reasonable to assume that the overall cell population represents a hyperoxia-tolerant variety that has survived hyperoxic insult over time by accepting 20% O_2 as a normoxic state. Although 20% O_2 then is normoxia for these cells, it is important to appreciate that this state may little resemble the state of cells or cellular responses *in vivo*.

How do cells alter their normoxic set point? Cellular O_2 sensing enables physiological adjustments to variations in tissue pO_2. Under basal conditions, cells are adjusted to an O_2 environment biologically read as normoxia (Khanna *et al.*, 2006). A sharp departure from that state triggers an O_2-sensitive biological response. The stabilization of hypoxia-inducible factor (HIF) signifies a robust biological readout of hypoxia. In the presence of sufficient O_2, HIF is hydroxylated and degraded. HIF prolyl hydroxylation is catalyzed by prolyl hydroxylase (PHD) isoenzymes PHD1, -2, and -3. Using cells stably transfected with an HIF reporter construct, the hypothesis that biological cells are capable of resetting their normoxic set point by O_2-sensitive changes in PHD expression has been tested (Khanna *et al.*, 2006). Exposure of a cell line adjusted to growing in 20% O_2 to 5% O_2 resulted in HIF-driven transactivation. However, the same cells adjusted to growing in 5%O_2 did not report hypoxia as read by HIF transactivation.

Notably, cells adjusted to growing in 30% O_2 reported hypoxia when acutely exposed to room air (20% O_2) culture conditions. When grown under elevated O_2 conditions, cells reset their normoxic set point upward by downregulating the expression of PHDs. When grown under low O_2 conditions, cells reset their normoxic set point downward by inducing the expression of PHDs. Exposure of mice *in vivo* to a hypoxic 10% O_2 environment lowered blood and brain pO_2. Such hypoxic exposure induced PHDs. Exposure of mice to a hyperoxic 50% O_2 ambience repressed the expression of PHD1–3, indicating that O_2-sensitive regulation of PHD expression is also effective *in vivo*. Studies employing knockdown of PHD expression reveal that O_2-sensitive regulation of PHD may contribute to tuning the normoxic set point in biological cells (Khanna *et al.*, 2006).

> Correction of wound hypoxia is required before tissues can respond to therapeutic interventions.

The state of tissue oxygenation is a major microenvironmental cue that is read by cells, integrated with other microenvironmental cues as a tissue responds to an extracellular signal. Although hypoxia has been studied extensively in the context of cell signaling, hyperoxia has been studied mostly in the context of oxygen toxicity. Both *in vitro* and *in vivo* studies with fibroblasts have revealed that hyperoxia may be a potent inducer of myofibroblast differentiation by turning on specific cell signaling events (Kuhn *et al.*, 2007; Roy *et al.*, 2003a,b, 2007, 2010; Sen *et al.*, 2006). In fibroblasts, hyperoxic insult causes growth arrest at the G2/M phase. This arrest is accompanied by induced expression of vimentin and α-smooth muscle actin, as well as by increased cellular contractility in a collagen matrix (Roy *et al.*, 2003a). Hyperoxia also enhances the stability of both Acta2 transcript and α-smooth muscle actin protein (Roy *et al.*, 2007). The morphological/cytoskeletal characteristics of fibroblasts observed in response to hyperoxic exposure match those of fibroblasts cultured at normoxia but treated with transforming growth factor β1 (TGF-β1), a classical inducer of fibroblast differentiation to myofibroblasts.

Interestingly, both hyperoxia and TGF-β signal through p38MAPK to cause fibroblast differentiation (Roy *et al.*, 2003a). In addition, TGF-β activation may be caused by hyperoxia-induced oxidation of the latency-associated peptide. Thus, hyperoxia may not only signal through the TGF-β pathway but also accentuate TGF-β signaling. Recent studies further support this relationship by demonstrating that in fibroblasts all three isoforms of TGF-β are induced by hyperoxia (Roy *et al.*, 2010). Deletion of any one or both of the activating protein-1 (AP-1) binding sites in the TGF-β reporter construct results in a loss of O_2 sensitivity, demonstrating that AP-1 confers O_2 sensitivity to TGF-β transcription. Fos-related AP-1 transcription factor (Fra-2) and apoptosis signal-regulating kinase-1 (Ask-1) have been identified as key mediators of AP-1-dependent hyperoxia-sensitive TGF-β transcription. Knockdown of Fra-2 significantly blunted hyperoxia-induced expression of TGF-β1 as well as TGF-β3 in fibroblasts (Roy *et al.*, 2010). Knockdown of Ask-1 blunted hyperoxia-induced Fra-2 gene expression and nuclear localization in fibroblasts. These observations point toward a central role of Ask-1 and Fra-2 in hyperoxia-inducible AP-1 activation and induction of TGF-β.

Transcriptome-wide profiling studies have identified hyperoxia-sensitive genes in fibroblasts and clustered them into functional groups (Roy *et al.*, 2003b). The p21–p53 axis has emerged as a key hyperoxia-inducible pathway in fibroblasts. Both p21 deficiency and knockdown blunt hyperoxia-induced Acta2 and smooth muscle actin expression. *In vivo*, reoxygenation-induced upregulation of Acta2 is abrogated completely in p21-deficient mice. Strikingly, overexpression of p21 alone induces differentiation of fibroblasts markedly under normoxic basal conditions. Overexpression of p21 alone induced transcription of α-smooth muscle actin by downregulating YB1 independent of TGF-β1. Thus, studies aimed at understanding the significance of O_2 tension have discovered p21 as a key signaling mediator that regulates the differentiation of fibroblasts to myofibroblasts (Roy *et al.*, 2003a, 2010).

The observations of Modarressi *et al.* (2010, this issue) establish a key role for

O_2 tension in driving wound contraction. A crucial contribution of this work is the demonstration that under hypoxic conditions compromised myofibroblast contraction is preceded by α-smooth muscle actin disassembly from stress fibers. Consistent with previous reports that the effects of changing O_2 tension on fibroblast differentiation are reversible (Roy *et al*., 2003a), Modarressi *et al*. demonstrate that the negative effects of hypoxia on fibroblast differentiation may be corrected by restoring normoxia. Interestingly, Modarressi *et al*. (2010) identify a facilitatory effect of mechanical stimulation in driving fibroblast differentiation; this effect was most pronounced under conditions of high oxygenation. This observation is relevant to negative pressure wound therapy, which involves mechanical stimulation and during which improved tissue oxygenation is followed by improved wound closure (Vikatmaa *et al*., 2008).

The wound literature is often confusing in terms of the net impact of hypoxia on wound closure (Sen, 2009). The abundance of literature (primarily related to tumor biology) demonstrating that hypoxia is a cue for angiogenesis has led many to erroneously conclude that hypoxia may be helpful for cutaneous wound healing. However, from a clinical standpoint we know that this is unlikely, because ischemic wounds are clearly hypoxic, yet refractory to closure. Acutely, hypoxia may help generate growth and repair factors necessary to lay the foundation for wound closure. However, unless there is sufficient oxygen (Sen, 2009), a good foundation for healing will not lead to closure. HIF-dependent hypoxia-inducible microRNA miR210 impairs wound epithelialization, a key aspect of overall wound closure (Biswas *et al*., 2010). Therefore, although stabilization of HIF may elicit angiogenic responses, the ultimate effect is to oppose wound closure by stalling wound re-epithelialization. The work by Modarressi *et al*. (2010) represents an important contribution to the field of wound healing, in which compelling evidence supports tissue hypoxia's conflict with wound closure. We are reminded that correction of wound tissue hypoxia is critical for wound tissue to respond to other therapeutic interventions.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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Lucky Number Seven: RNase 7 Can Prevent *Staphylococcus aureus* Skin Colonization

John S. Cho[1], Caiyun Xuan[1] and Lloyd S. Miller[1]

***Staphylococcus aureus* colonization is a major risk factor for infection. In this issue, Simanski *et al.* demonstrate that the antimicrobial peptide RNase 7 is essential for preventing *S. aureus* colonization in human skin. These findings suggest that therapeutic interventions aimed at targeting RNase 7 production in the skin may be a novel strategy to protect against *S. aureus* infections.**

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Antimicrobial peptides and skin host defense

Human skin presents both a physical and an immunological barrier against invading microbial pathogens. In addition to contributing to the physical barrier, the stratum corneum contains antimicrobial peptides that act as a first line of defense against pathogenic microbial colonization and infection by pathogens such as bacteria, fungi, and viruses (Lai and Gallo, 2009).

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