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AUTHOR'S VIEWS

Survival of the resilient: Mechano-adaptation of circulating tumor cells to fluid shear stress

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ABSTRACT

During metastasis, cancer cells traverse the circulation to reach distant organs. Conventionally, this journey has been regarded as mechanically destructive to circulating tumor cells from solid tissues. We have recently shown that cancer cells from diverse tissues actively resist destruction by fluid shear stress through a mechano-adaptive RhoA-actomyosin mechanism.

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The mechanobiology of cancer has yielded rich insights into how cancer cells respond to the solid state mechanics of the tumor microenvironment, for example, increased matrix stiffness, as well as compressive stress, can elevate the invasive phenotype of cancer cells.[1] Fluid shear stress (FSS), which results from fluid velocity gradient in the lumen of a vessel produced by the friction of fluid flow at the vessel walls,[2] is also one of the major physical forces that cancer cells will experience and is known to produce important biological effects in many cell types.[3] In solid tumors, cancer cells experience low magnitude FSS from interstitial flows which also contribute to important transport phenomena in tumors.[1] Cells exposed to interstitial flows also remain attached to the extracellular matrix and other cells. In marked contrast, once cancer cells enter the circulation and become circulating tumor cells (CTCs), they experience FSS orders-of-magnitude greater than cancer cells in the solid tumor microenvironment, and are in a detached state.[2]

What happens to cancer cells in this liquid microenvironment of the circulation is poorly understood, as the rarity of CTCs (~1-10 CTC/10^9 blood cells) and the vastness of the circulatory system (10^5 km) pose great experimental challenges to monitoring the fate of CTCs.[2] It has been remarked that CTCs subjected to FSS are mechanically fragile and many are destroyed.[4] Although this might be intuitive and supported by the fact that most CTCs do not generate clinically relevant metastases, direct evidence that CTCs are mechanically fragile when exposed to FSS has been lacking. Moreover, efforts to track the fates of experimental CTCs in mice have shown that they survive their initial exposure to the circulation with high efficiency.[5]

In an attempt to determine the effect of FSS on CTCs, research has been performed evaluating the effects of FSS on cancer cells *in vitro;* however, there is no currently available *in vitro* system that fully recapitulates the fluid dynamics observed in mammalian circulatory systems.[2] Some have developed continuous flow circuits that can approximate mean arterial or venous levels of FSS, reviewed in.[2] However, a limitation of these models is that cancer cells are often subjected to FSS continuously for hours, whereas CTCs spend brief periods, seconds-to-minutes, in free circulation and much longer periods of time trapped in the microcirculation.[6] We developed an *in vitro* model employing a needle and syringe to expose cancer cells to repeated "pulses" of high level FSS.[7] In this model, we deliver millisecond pulses of FSS levels that are at or above the highest levels that exist physiologically, such as those CTCs would experience in the turbulent flows that briefly develop around heart valves. This model too is limited as it does not evaluate longer duration of low-level FSS on cancer cells. Utilizing this model, we were surprised to find that cancer cells from diverse tissue origins are relatively resistant to destruction by FSS as compared to non-transformed epithelial cells derived from those same tissues.[7] Early attempts to understand the mechanism-(s) underlying FSS resistance have demonstrated the importance of cellular stiffness, such as actin dynamics, Rho Kinase activity or nuclear structure[7-9] (Figure 1). However, a detailed understanding of this mechanism of FSS was elusive and much of this work relied on cell lines and how it was relevant to CTCs was unclear.

In our recent study we demonstrated that exposure to FSS *in vitro* results in the activation of ras homolog family member A (RHOA) in cancer cells (Figure 1).[10] FSS exposure also led to both a formin-dependent increase in cortical F-actin levels and an increase in activation of myosin, consistent with the activation of these cytoskeletal master regulators, that prevents damage to the plasma membrane induced by FSS (Figure 1).[10] Inhibiting the mechano-adaptive response through the RHOA-actomyosin network led to an increase in the fraction of cells destroyed by both *in vitro* applied FSS and hemodynamic FSS, a decrease in the CTC burden of orthotopically injected mice, and a delay in the onset of

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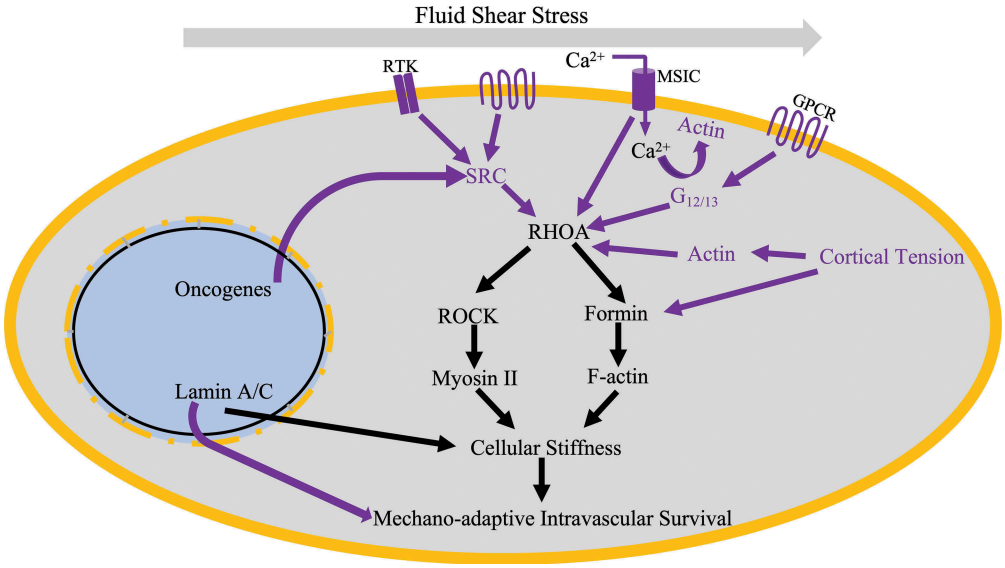

Figure 1. Mechano-adaptation to fluid shear stress exposure. Proposed mechanisms for adaptation to shear stress exposure with known components outlined in black and known mechano-sensitive signaling components that have been shown alter *RHOA* or actin cytoskeleton dynamics in purple. The pathways outlined in purple are potentially activated by fluid shear stress exposure. (MSIC = mechanosensitive ion channel; RTK = receptor tyrosine kinase; GPCR = G-protein coupled receptor; ROCK = Rho Kinase; $G_{12/13}$ = G-alpha protein 12 or 13; SRC-Src Kinase).

metastatic disease using an experimental metastasis model.[10] Importantly, we also showed that FSS resistance is a property of cancer cells directly isolated from genetically-engineered mouse primary tumors and patient-derived xenografts, demonstrating that FSS resistance observed in cancer cells is not a product of *in vitro* culture or metastatic selection. Collectively these data demonstrate the importance of mechano-adaptive FSS resistance for the survival of CTCs. Thus, mechanical destruction of CTCs by hemodynamic forces is not likely to be a significant contributing factor to the highly inefficient process by which CTCs may become metastatic colonies.

Interestingly, non-transformed cells do not undergo these mechano-adaptive responses when exposed to FSS while in suspension.[9,10] Moreover, cellular transformation by deletion of tumor suppressor genes or expression of oncogenes confers FSS resistance.[7,10] Whether transformation enables FSS resistance via altering the regulation of cellular mechanics or upregulation of mechanosensitive proteins is unknown. It is also unclear how cancer cells sense FSS while in suspension, whether this involves mechanoreceptors or if membrane stretching acts on the cortical F-actin network to enable cancer cells to sense FSS (Figure 1). What is clear is that the RHOA-dependent mechanism isn't the only one driving FSS resistance, as inhibiting the RHOA-actomyosin pathway doesn't reduce FSS resistance to the level of non-transformed cells. Addressing these fundamental questions is essential since mechano-adaptation to FSS has implications for downstream events in metastasis. Finally, the potential of inhibiting FSS resistance as a therapeutic strategy and how this might be employed clinically remains unknown.

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