Elucidating the Role of Microenvironmental Cues on Melanoma Drug Resistance

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Statement of Purpose: Melanoma is notoriously drug resistant and accounts for approximately 75% of all skin cancer related deaths[1]. Clinically, tissue stiffness has been linked to malignancy, leading to the theory that the local environment plays a crucial role in regulating disease progression for many forms of cancer. Both matrix stiffness and receptor binding have been correlated with malignant morphology in breast cancer[2,3], but the same has not yet been determined for melanoma. Due to its prevalence with outside-in signaling and high mutation rate, the mitogen-activated protein kinase ERK kinase (MEK) pathway is both a mechanism through which cells may probe the local environment and an attractive target for cancer therapy[4]. As a result, MEK pathway inhibitors were developed, and in 2011 Vemurafenib (Genentech, San Francisco, CA) was approved by the FDA for metastatic melanoma treatment. Here, we focus on Vemurafenib, an inhibitor of B-rapidly accelerated fibrosarcoma (B-RAF), as a model drug to investigate melanoma responses to this treatment while systematically altering the local environment. The goal of this work is to determine how microenvironmental factors such as substrate stiffness and ligand binding contribute to melanoma behavior and resistance to B-RAF inhibition. We hypothesize that mature focal adhesion formation and Filamin A expression, an important component of mechanosensing, may abrogate drug-induced effects when melanoma cells are cultured on softer substrates such as poly(ethylene glycol) (PEG)-based hydrogels.

Methods: Four-arm norbornene-functionalized PEG was crosslinked with a di-thiol-containing enzymatically degradable peptide (KCGPQGIWGQCK) via a light-induced thiol-ene polymerization (365 nm, 1.5 mW/cm^2). The pendant peptide CRGDS was incorporated in the hydrogels to control cell attachment and spreading. Using this system, the modulus and adhesivity was widely tuned. Hydrogels were formed on thiol-functionalized coverslips, followed by cell seeding. Four different melanoma cell lines were assessed: one derived from the radial growth phase (WM35), one from the vertical growth phase (WM115), and two metastatic cell lines (WM239A and A375). Each cell type has been previously assessed for a B-RAF mutation, which should render these cells susceptible to MEK pathway inhibition. The B-RAF inhibitor PLX4032, clinically known as Vemurafenib, (ChemieTek, Indianapolis, IN) was chosen due to its proven efficacy in the clinic. Metabolic activity was measured with CellTiter-Glo (Promega, Madison, WI), DNA content was assessed by PicoGreen (Life Technologies, Grand Island, NY), and immunostaining for β1-integrin was performed. Western blot analysis was used to analyze the levels of pERK ½, pFAK^{Y397} and Filamin A.

Results: Using metabolic activity as a measure for cytotoxic effects, WM115 and WM239A cells responded similarly to PLX4032 on TCPS (tissue culture treated polystyrene) or on softer gel substrates. Interestingly, the radial growth phase melanoma cells (WM35) appeared to survive well in the presence or absence of the inhibitor regardless of what substrate they were cultured on. A375 cells were more sensitive but appeared to survive better on stiff hydrogel surfaces compared to TCPS when treated with PLX4032. Based on DNA content analysis, the A375 cells suffered significant cell death in addition

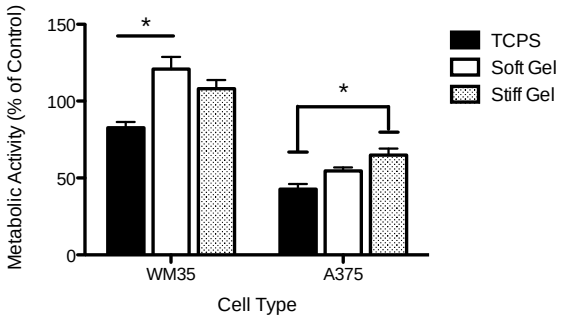

Figure 1. Metabolic activity of melanoma cells on TCPS or soft and stiff hydrogels**.** *, $p < 0.05$.

to decreased metabolic activity. Qualitatively, A375 cells appeared more spread on TCPS compared to either gel formulation, which could contribute to differences in metabolic activity. To better understand this phenomenon, Western blot analysis was performed to determine the presence of Filamin A. We found A375 cells expressed Filamin A, and B-RAF inhibition decreased Filamin A expression. WM35 cells showed low basal levels of Filamin A, but expression increased with B-RAF inhibition. Analysis of pFAK^{Y397} was inconclusive.

Conclusions: Our results suggest A375 cells are more sensitive to matricellular signals and resist drug treatment, based on increased metabolic activity on hydrogels, altered morphology, and Filamin A expression. While Filamin A may ultimately play an important role in how melanoma cells respond to B-RAF inhibition, it cannot be assumed to be the sole effector. Currently, caspase 3 activation and proliferation are being studied to determine how substrate stiffness alters these processes with drug treatment. Lastly, studies varying the concentration of the adhesion peptide RGDS are planned to elucidate how adhesivity affects melanoma's sensitivity to PLX4032 treatment.

References:

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