Matrix elasticity regulates melanoma cell survival

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Statement of Purpose: Melanoma is an aggressive form of skin cancer that tends to be highly drug resistant in later stages of progression, but the mechanisms that allow its escape from drug treatment are not completely understood[1]. The AKT signaling pathway is commonly associated with cell survival, and specifically, elevated levels of the isoform AKT3 have been associated with drug resistant melanoma[2]. Activation of AKT, i.e. phosphorylation (pAKT), is matrix adhesion dependent and protects melanoma from anoikis[3]. One factor that may influence such signaling is the microenvironment, which is thought to play a significant role in tumor cell survival and proliferation; matrix elasticity, in particular, has been correlated with malignancy and tumor progression in breast cancer[4]. However, it is unclear what role the substrate elasticity plays in melanoma drug responsiveness. To investigate how matrix elasticity influences melanoma cell survival, we utilized poly(ethylene glycol) (PEG) hydrogels to tune the matrix modulus. We investigated the survival of two melanoma cell lines derived from different stages (radial growth phase WM35 and metastatic A375) in response to pharmacological inhibition of B-rapidly accelerated fibrosarcoma (BRAF) with the drug PLX4032 (Vemurafenib, Genentech, San Francisco, CA). The goal of this work is to better understand the molecular mechanisms of microenvironmental-induced changes in melanoma that may contribute to a more drug resistant phenotype. Based on our initial findings, we hypothesize that increased survival signaling through PI3K/AKT leads to survival of A375 cells on soft substrates. Additionally, we hypothesize that the WM35 cells have decreased PI3K/AKT signaling as a result of reduced cell-matrix interactions on more compliant substrates.

Methods: Hydrogels were formed via the photoinitiated (365 nm, 1.5 mW/cm^2) thiol-ene "click" reaction. Here, 4-arm norbornene-functionalized PEG was crosslinked with a bi-functional cysteine-containing MMP-degradable peptide (KCGPQGIWGQCK). The modulus of the hydrogels was varied by changing the crosslink density, and the fibronectin-derived CRGDS pendant peptide was incorporated at 1.5 mM to promote cell adhesion. Hydrogels were formed on thiolated coverslips and then seeded with human melanoma cells (WM35 or A375). The BRAF inhibitor PLX4032 (ChemieTek, Indianapolis, IN) was used for all studies, as both cell lines have been previously assessed for a BRAF mutation that would make them susceptible to PLX4032 treatment. Apoptosis was measured using the EnzCheck Caspase 3 Assay (Life Technologies, Grand Island, NY) and western blot analysis was used to assess levels of pERK, pFAK^{Y397}, and pAKT. Immunostaining for the focal adhesion protein paxillin was used to visualize cell-matrix interactions.

Results: Hydrogels with moduli from 0.6 to 13 kPa (E, Young's modulus) were used to assess the effects of matrix elasticity on melanoma cell responses to PLX4032. On compliant substrates, WM35 cells had smaller focal adhesions and exhibited increased PLX4032-induced apoptosis as compared to stiffer substrates. In contrast, A375 cell function appeared to be stiffness-independent, with no statistical difference in focal adhesion size or apoptosis due to drug treatment. To investigate the mechanism that regulates this differential response with substrate stiffness, basal levels of pAKT were measured to determine whether increased PI3K/AKT signaling might play a role in increased survival. Unexpectedly, AKT activity (pAKT/AKT) in A375 cells increased on more compliant substrates, while lower activity levels were observed in the WM35 cells. Analysis of pERK and pFAK^{Y397} is underway.

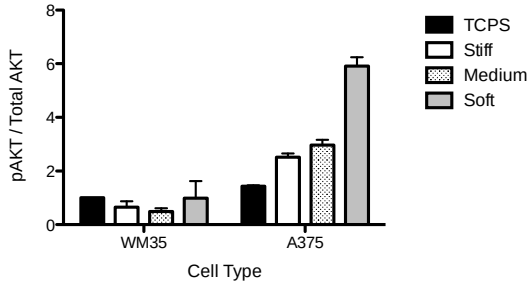

Figure 1. Levels of pAKT/total AKT in WM35 and A375 cells (n=2). Normalized to WM35 TCPS samples.

Conclusions: These results suggest that A375 cells are less dependent on matrix elasticity when treated with PLX4032 for cell survival, but the A375s appear to respond to soft substrates with increased signaling through pAKT. The WM35 cells have lower levels of pAKT, smaller focal adhesions, and increased apoptosis on soft gel substrates. Future studies aim to determine if actin stress fibers or mature focal adhesion formation induce increased AKT or ERK signaling in WM35 cells on TCPS. We plan to investigate other possible pathways that may lead to increased AKT levels and assess whether AKT may be suppressing apoptosis or increasing proliferation in A375 cells to promote viability.

References:

1. Soengas MS. Oncogene. 2003;22:3138-3151.
2. Shao Y. Cancer Res. 2010;79:6670-6681.
3. Boisvert-Adamo K. Oncogene. 2006;25:4848-4856.
4. Paszek MJ. Cancer Cell. 2005;8:241-254.