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Activation of the Mitochondrial Fragmentation Protein DRP1 Correlates with BRAF^{V600E} Melanoma

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To The Editor

The shape of the mitochondrial network results from the cumulative activity of two opposing processes: fusion and fission (Mishra and Chan, 2014). These processes collaborate to ensure homeostatic maintenance of mitochondrial function, cellular bioenergetics, and commitment to mitosis (Nasrallah and Horvath, 2014). While the contributions of aberrant mitochondrial dynamics in neurodegenerative and cardiometabolic diseases are established, little is known about the contribution of mitochondrial dynamics in cancer development, prognosis, or treatment.

Recently, a role for dynamin related protein 1 (DRP1) was revealed in oncogenic RAS-induced cellular transformation, and in cellular responses to oncogenic MAPK inhibition

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(*e.g.*, $BRAF^{V600E}$ inhibition with PLX-4032)(Bollag *et al*., 2010; Serasinghe *et al*., 2015). DRP1 is a large cytosolic GTPase that induces fission of the mitochondrial network (Yoon *et al.*, 2001; Smirnova *et al*., 2001). For example, when DRP1 is phosphorylated at serine 616 ($DRP1^{S616℗}$), DRP1 localizes to mitochondria, undergoes oligomerization, and initiates membrane scission (Mishra and Chan, 2014). $DRP1^{S616℗}$ is directly induced by ERK1/2 within the $BRAF^{V600E}$ pathway leading to chronic mitochondrial fission, cancer-associated mitochondrial dysfunction, and resistance to targeted therapies (Serasinghe *et al*., 2015). In melanoma, $DRP1^{S616℗}$ status dichotomized wild type BRAF ($BRAF^{Wt}$) from $BRAF^{V600E}$ disease, suggesting a mechanistic contribution of $DRP1^{S616℗}$ in $BRAF^{V600E}$ melanoma (Serasinghe *et al*., 2015). Based on these observations, we were interested in determining if $DRP1^{S616℗}$ was prevalent in all $BRAF^{V600E}$ skin lesions (*e.g.*, nevi), or if $DRP1^{S616℗}$ was indicative of $BRAF^{V600E}$ melanoma.

To investigate this question, we performed IHC for the $BRAF^{V600E}$ and $DRP1^{S616℗}$ status on a cohort of tissues. Benign nevi (68 samples; Figures 1a & S1a), dysplastic nevi (40 samples; Figures 1b & S1b), primary melanomas (187 samples; Figures 1c & S1c), and nevi derived from patients eventually diagnosed with melanoma (46 sets; Figure 1d) were stained. $BRAF^{V600E}$ and $DRP1^{S616℗}$ scoring methods were developed (0, 1+ = negative; 2+, 3+ = positive) based on standard histopathological analyses within the Mount Sinai Medical Center and relevant literature (Figures S1a-c) (Pearlstein *et al*., 2014; Serasinghe *et al*., 2015). As control, we examined tissues stained with no primary antibody and rabbit IgG to ensure specificity (Figures S2a-d & S3a-b); and we also examined total DRP1, which was minimally expressed in normal skin, benign nevi, $BRAF^{Wt}$ melanoma, and $BRAF^{V600E}$ melanoma (Figures S2a-d & S3a-b).

Our benign nevi collection demonstrated no dysplasia at the time of diagnosis, had no known relationship to melanoma, and $DRP1^{S616℗}$ was not significantly related to BRAF status (Figures 1a). While melanoma progression is not absolutely understood, dysplastic nevi are often considered precursors to disease and increase the risk of developing melanoma (Goldstein and Tucker, 2013). Indeed, analysis of a dysplastic nevi collection from the Mount Sinai Dermatopathology Division which contained a subset of tissues derived from patients eventually diagnosed with melanoma revealed that approximately 79.3% (23/29 cases) of tissues that are $DRP1^{S616℗}$ positive are also $BRAF^{V600E}$, and 92% (23/25 cases) of $BRAF^{V600E}$ dysplastic nevi display $DRP1^{S616℗}$ (Figure 1b). Within the melanoma panel (59 $BRAF^{Wt}$, 128 $BRAF^{V600E}$), we observed $DRP1^{S616℗}$ in 91 samples; the vast majority (87/91 cases, 95.6%) was in $BRAF^{V600E}$ tumors (Figure 1c). In contrast, only 4 out of 59 $BRAF^{Wt}$ tumors were positive for $DRP1^{S616℗}$. Fisher's Exact ($p<0.0001$) and Chi-Squared ($p<0.0001$) analyses revealed that these relationships are highly significant. We also analyzed an additional larger cohort of nevi (containing benign and dysplastic) that are all matched to patients with melanoma, and similar relationships were obtained (Figure 1d). Together, these data suggest that $DRP1^{S616℗}$ is significantly related to $BRAF^{V600E}$ status in dysplastic nevi and human melanoma, with correlations most striking in $BRAF^{V600E}$ melanoma.

Literature and the above data suggest that $DRP1^{S616℗}$ may contribute to the survival of $BRAF^{V600E}$ disease (Serasinghe *et al*., 2015). Indeed, silencing oncogenic MAPK signaling

via the pharmacological inhibition of $BRAF^{V600E}$ or MEK (with PLX-4032 or GSK-1120212, respectively) decreased $DRP1^{S616Ⓟ}$ by western blot and immunofluorescence, but not $DRP1^{Total}$, in $BRAF^{V600E}$ melanoma cells (Figures 2a & S4a). The oncogenic MAPK pathway often reactivates following the inhibition of $BRAF^{V600E}$ or MEK, and this confounds interpreting a direct pro-survival role for $DRP1^{S616Ⓟ}$ (Holderfield *et al*., 2014). Therefore, we examined $DRP1^{S616Ⓟ}$ contributions in the proliferation and survival of $BRAF^{V600E}$ melanoma cell lines by DRP1 loss of function experiments using RNAi and a small molecule (mDIVI-1) that durably inhibits mitochondrial fission by blocking the DRP1 GTPase (Cassidy-Stone *et al*., 2008). A375 cells were infected with RNAi lentivirus against *Drp1*, and monitored for proliferation. Loss of *Drp1* expression correlated with decreased proliferation and clonogenic survival (Figures 2b-e). Next, A375 cells were treated with mDIVI-1, evaluated by fluorescent microscopy for expected changes to mitochondrial shape (*i.e.*, mitochondrial fusion = DRP1 inhibition), and then scored for apoptotic responses. Indeed, the inhibition of DRP1 function by mDIVI-1 led to a marked decrease in DRP1-dependent mitochondrial fission (Figure 2f) and dose-dependent apoptosis (Figure 2g). In contrast, the $BRAF^{Wt}$ melanoma line MeWo displayed minimal $DRP1^{S616Ⓟ}$ and blunted pro-apoptotic responses to mDIVI-1 treatment (Figures S5a-b). We also treated these cells with staurosporine to ensure they had intact pro-apoptotic signaling (Figure S5c).

Altogether, these data suggest that the induction of $DRP1^{S616Ⓟ}$ in dysplastic nevi (and nevi derived from patients eventually diagnosed with melanoma) and primary melanoma is a potential contributing factor to $BRAF^{V600E}$ disease; and examining $DRP1^{S616Ⓟ}$ status may be a useful progression biomarker along with $BRAF^{V600E}$ to determine which lesions are most likely to develop into disease. $DRP1^{S616Ⓟ}$ is undetectable in normal skin, and the frequency of genomic alterations to *DRP1* is only ∼10% in cancer (Figures S2a-b & S6a-b) (Cerami *et al*., 2012; Gao *et al*., 2013, Serasinghe *et al*., 2015). However, the activation of DRP1 by oncogenic MAPK signaling that occurs during cellular transformation is regulated in the majority of samples (Serasinghe *et al*., 2015). In addition, $DRP1^{S616Ⓟ}$ is markedly enhanced in $BRAF^{V600E}$ positive dysplastic nevi and melanomas. This activation correlates with the survival of $BRAF^{V600E}$ cancer cells following treatment with targeted therapies (Serasinghe *et al*., 2015). While there is also a subset of $BRAF^{V600E}$ positive lesions that are negative for $DRP1^{S616Ⓟ}$ (Figure 1c), recent studies suggest there are alternative mechanisms to induce mitochondrial hyper-fragmentation and subsequent apoptotic resistance through the mitochondrial dynamics machinery (Renault *et al*., 2015). Collectively, these efforts suggest that studying DRP1, and potentially other proteins involved in orchestrating mitochondrial dynamics and function, may offer a unique perspective to better understand melanoma development, diagnosis, and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BRAF	v-Raf murine sarcoma viral oncogene homolog B
DRP1	dynamin related protein 1
ERK	extracellular signal-regulated kinase
FFPE	formalin-fixed paraffin embedded
FITC	fluorescein isothiocyanate
GTP	guanosine-5′-triphosphosphate
IHC	immunohistochemistry
MEK	mitogen-activated protein kinase/ERK kinase

RAS rat sarcoma

a

BRAF Status (Wt / V600E)	DRP1$^{S616Ⓟ}$ (+ / -)	Benign Nevi # / 68 (%)	Fisher's Exact	Chi-Squared (X^2)
$BRAF^{Wt}$	DRP1$^{S616Ⓟ}$(-)	5 (7.4%)	*p = 0.4291*	*p = 0.2646*
$BRAF^{Wt}$	DRP1$^{S616Ⓟ}$(+)	2 (2.9%)		
$BRAF^{V600E}$	DRP1$^{S616Ⓟ}$(-)	30 (44.1%)		
$BRAF^{V600E}$	DRP1$^{S616Ⓟ}$(+)	31 (45.6%)		

b

BRAF Status (Wt / V600E)	DRP1$^{S616Ⓟ}$ (+ / -)	Dysplastic Nevi # / 40 (%)	Fisher's Exact	Chi-Squared (X^2)
$BRAF^{Wt}$	DRP1$^{S616Ⓟ}$(-)	9 (22.5%)	*p = 0.0007*	*p = 0.0004*
$BRAF^{Wt}$	DRP1$^{S616Ⓟ}$(+)	6 (15.0%)		
$BRAF^{V600E}$	DRP1$^{S616Ⓟ}$(-)	2 (5.0%)		
$BRAF^{V600E}$	DRP1$^{S616Ⓟ}$(+)	23 (57.5%)		

c

BRAF Status (Wt / V600E)	DRP1$^{S616Ⓟ}$ (+ / -)	Melanoma # / 187 (%)	Fisher's Exact	Chi-Squared (X^2)
$BRAF^{Wt}$	DRP1$^{S616Ⓟ}$(-)	55 (29.4%)	*p < 0.0001*	*p < 0.0001*
$BRAF^{Wt}$	DRP1$^{S616Ⓟ}$(+)	4 (2.1%)		
$BRAF^{V600E}$	DRP1$^{S616Ⓟ}$(-)	41 (21.9%)		
$BRAF^{V600E}$	DRP1$^{S616Ⓟ}$(+)	87 (46.5%)		

d

BRAF Status (Wt / V600E)	DRP1$^{S616Ⓟ}$ (+ / -)	Matched Nevi # / 46 (%)	Fisher's Exact	Chi-Squared (X^2)
$BRAF^{Wt}$	DRP1$^{S616Ⓟ}$(-)	5 (10.9%)	*p = 0.0249*	*p = 0.0131*
$BRAF^{Wt}$	DRP1$^{S616Ⓟ}$(+)	6 (13.0%)		
$BRAF^{V600E}$	DRP1$^{S616Ⓟ}$(-)	4 (8.7%)		
$BRAF^{V600E}$	DRP1$^{S616Ⓟ}$(+)	31 (67.4%)		

Figure 1. Increased DRP1$^{S616Ⓟ}$ is associated with the incidence of BRAF^{V600E} melanoma

(ad) IHC was performed to detect the status of BRAF^{V600E} and DRP1$^{S616Ⓟ}$ in benign nevi (68 samples, *a*), dysplastic nevi (40 samples, *b*), primary melanoma (187 samples, *c*), and nevi from patients that developed melanoma (46 samples, *d*). Fisher's Exact and Chi-Squared Tests determined statistical significance.

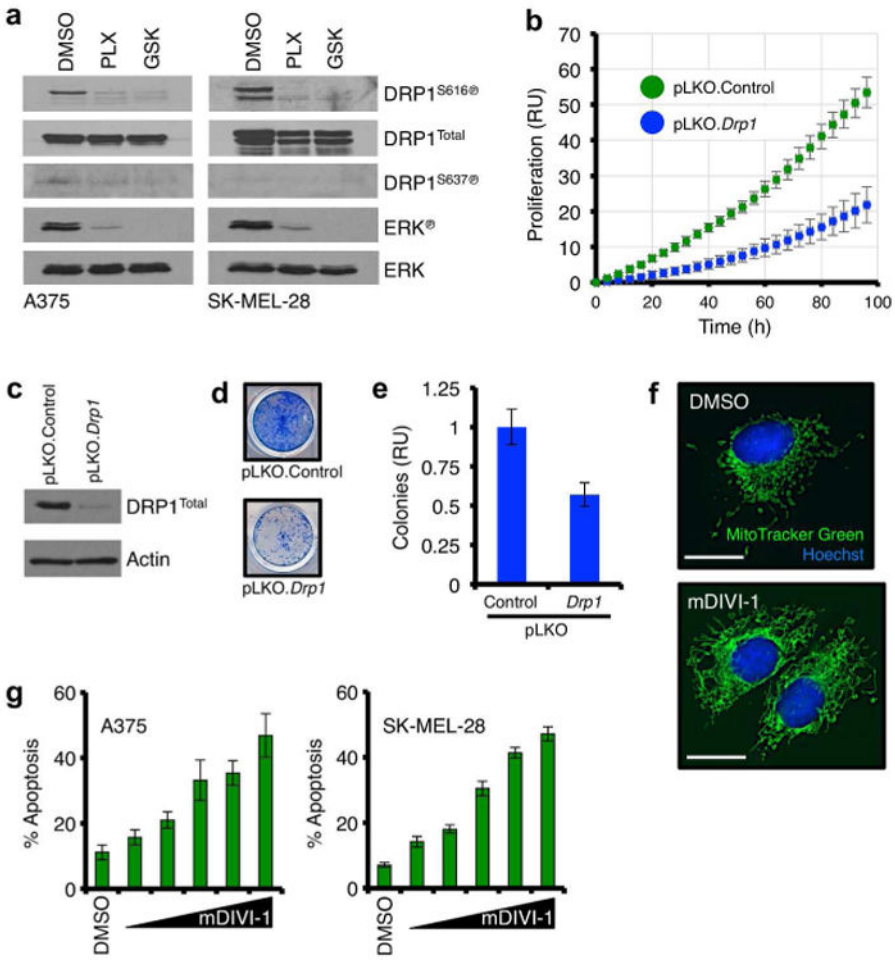

Figure 2. Inhibition of DRP1 suppresses BRAF^{V600E} melanoma cell growth and survival

(a) A375 and SK-MEL-28 cells were treated with PLX-4032 (1 μM) or GSK-1120212 (10 nM) for 8 hours, and lysates were western blotted for indicated proteins. ERKⓅ is shown as a positive control for drug sensitivity. Multiple DRP1 isoforms explain the presence of additional bands in the SK-MEL-28 DRP1 blots. **(b)** A375 cells were infected with control or *Drp1* RNAi, and proliferation was quantified for 96 hours. **(c)** A375 cells were infected with control or *Drp1* RNAi, and lysates were western blotted for indicated proteins. **(d)** A375 cells were infected with control or *Drp1* RNAi, cultured for 12 days, and stained. **(e)** Colony formation in *d* was quantified. **(f)** A375 cells were treated with mDIVI-1 (10 μM) for 8 hours, and loaded with MitoTracker Green and Hoechst 33342 before live cell imaging. **(g)** A375 and SK-MEL-28 cells were treated with mDIVI-1 (0, 5, 10, 25, 50, 100 μM) for 48 hours before AnnexinV-FITC analysis. All data are representative of at least triplicate experiments, and reported as ± S.D., as required. Scale bars = 25 μm.