p38 isoform signaling in human cutaneous skin melanoma: insights from in vitro studies and database mining analyses

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INTRODUCTION

Skin cutaneous melanoma (SKCM) accounts for 75% of skin cancer deaths, with incidence rates continuing to rise alarmingly. Therapeutic strategies for advanced melanoma, such as targeted therapies and immunotherapies, are rapidly emerging, but drug resistance and toxicity remain challenges for many patients. Understanding the underlying molecular mechanisms in SKCM is key to identifying novel biomarkers critical for predicting treatment response and discovering new targeted therapy approaches. The p38 protein kinases coordinate adaptive cellular responses to extracellular stimuli and modulate important processes dysregulated in tumorigenesis, such as proliferation, differentiation and survival. The mammalian p38 MAPK family includes p38α, p38β, p38γ, and p38δ isoforms, encoded by *MAPK14*, *MAPK11*, *MAPK12*, and *MAPK13* genes, respectively. Although p38 signaling is of potential importance in melanoma, the isoform-specific functions of the p38s in SKCM are largely unelucidated.

METHODS

Here we examined the effects of pharmacologic and RNA interference-mediated inhibition of p38 isoforms in human melanoma cell lines A375 and WM164 using colony formation assay. SB203580 was used for p38α/p38β inhibition and Compound 62 for pan-p38 inhibition.

We also analyzed the gene expression, prognostic value, and clinical correlations of the p38 isoforms in The Cancer Genome Atlas SKCM sample datasets, utilizing bioinformatic tools such as GEPIA, LinkedOmics, TIMER, and GSCALite.

RESULTS

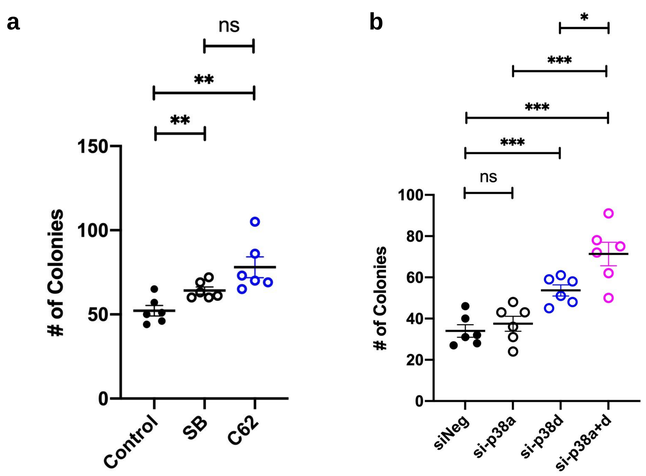

Figure 1. Enhanced colony formation ability of the A375 human melanoma cell line with p38 isoform inhibition. (**a**) Pharmacologic inhibition (**b**) Small interfering RNA-mediated inhibition. p38α/p38β inhibition with SB203580, pan-p38 inhibition with Compound 62, or a simultaneous knockdown of both p38α and p38δ enhanced colony formation ability, highlighting both specific and redundant ***roles for p38 isoforms in negative regulation of human melanoma cell survival***. The increase in colony number reflects survival of melanoma cells.

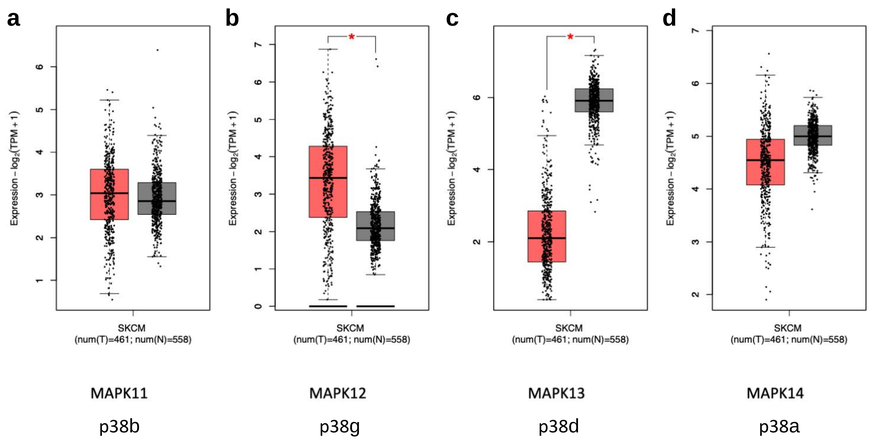

Figure 2. The expression of p38 isoforms in SKCM (Gene Expression Profiling Interactive Analysis) GEPIA uses RNA sequencing patient level data for tumor and normal differential expression, correlation to pathologic stage, and patient survival analysis The mRNA expression of MAPK13 was significantly lower in SKCM tissue (in red) than in normal tissue (**c**), and mRNA of MAPK12 was significantly higher in tumor (in red) versus normal tissue (**b**). Although gene expression does not capture post-translational modifications that can affect the phenotypic result, these results hint at a ***tumor-promoting role for p38γ and a tumor-suppressing role for p38δ in cutaneous melanoma.***

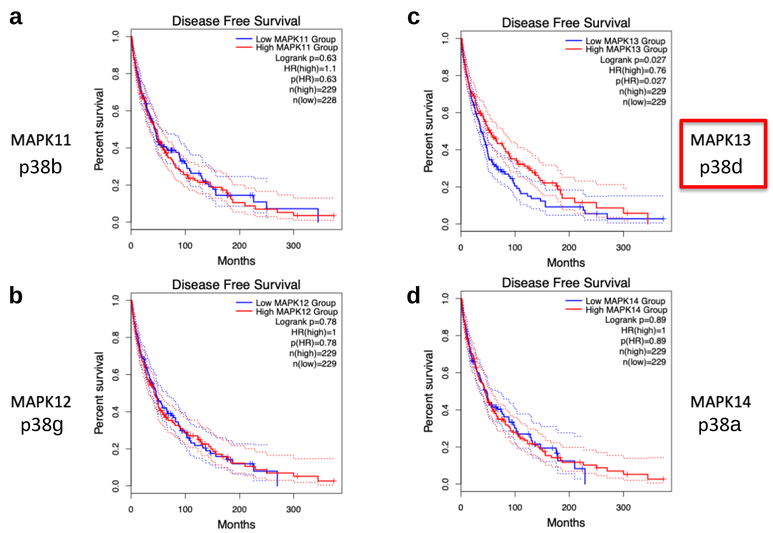

Figure 3. Prognostic value of p38 isoforms in SKCM (GEPIA) ***The disease-free survival of SKCM patients with high p38δ/MAPK13 level was better compared with patients with low p38δ/MAPK13 levels, consistent with a tumor-suppressing role for p38δ in cutaneous melanoma (c)*** No significant differences were appreciated for the other p38 isoforms (**a,b,d**).

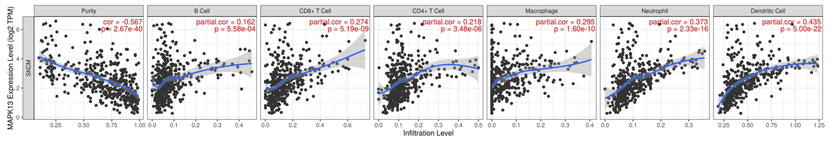

Figure 4. Correlation of p38δ with immune cell infiltrates in SKCM tissue (Tumor Immune Estimation Resource) TIMER assesses clinical impact of various immune cells by correlating gene expression of the respective MAPKs with the abundance of immune cell infiltrates and immune biomarkers in SKCM tissue. Tumor purity is a major confounding factor in this analysis, with most immune cell types negatively correlated with tumor purity. Therefore, the partial Spearman's correlation was used to adjust for tumor purity for this association analysis. ***MAPK13 was positively correlated with B cells, CD4+ T cells, macrophages, neutrophils and dendritic cells in SKCM tissue, supporting a tumor-suppressing role for p38δ in cutaneous melanoma.***

Immune Cells	Biomarkers	Correlation Adjusted for Purity for MAPK11		Correlation Adjusted for Purity for MAPK12		Correlation Adjusted for Purity for MAPK13		Correlation Adjusted for Purity for MAPK14	
T cell exhaustion	PD-1 (PDCD1)	-0.029	NS	-0.116	*	0.260	**	0.064	NS
	CTLA4	0.035	NS	0.091	NS	0.170	*	0.028	NS
	LAG3	-0.019	NS	-0.078	NS	0.250	**	0.061	NS
	TIM-3 (HAVCR2)	0.029	NS	-0.005	NS	0.387	***	0.271	**
	GZMB	0.038	NS	-0.040	NS	0.294	***	0.009	NS

Table 1. Correlation of p38 isoforms with immune biomarkers in SKCM tissue (TIMER) ***All investigated biomarkers of T cell exhaustion (PD-1, CTLA4, LAG3, TIM-3, GZMB) were significantly positively correlated with MAPK13 level in SKCM tissue. The association of T cell exhaustion biomarkers with MAPK13 suggests that p38δ can be a target for therapeutic inhibition alongside immune checkpoint blockade.*** Additionally, higher MAPK13 expression was significantly associated with a higher level of immune biomarkers for the cell populations of significance in the immune infiltration data of Figure 4 (not shown here), reinforcing ***a tumor-suppressing role for p38δ in cutaneous melanoma.***

DISCUSSION

Our colony assay results highlight both specific and redundant roles for p38 isoforms in negative regulation of human melanoma cell survival. Although p38δ knockdown increased human melanoma cell survival *in vitro*, simultaneous knockdown of p38α and p38δ resulted in significantly greater cell survival. Our large database genomics results complemented the *in vitro* data and provided new insights, showing that p38δ was downregulated, while p38γ expression was upregulated in SKCM. In addition, a low level of p38δ correlated with worse disease-free survival. We report a novel melanoma-promoting role for p38γ and reinforce a melanoma-suppressing role for p38δ. Furthermore, higher p38δ was correlated with increased immune cell infiltration, including CD8+ T cells and dendritic cells, and increased T cell exhaustion, suggesting that targeting p38δ in the SKCM tumor microenvironment may stimulate antitumor immunity. Our study highlights the potential paths for translational research efforts.

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