Patterns of PI3K/Akt/mTOR, Ras/Raf/MEK/ERK and Jak2/Stat3 pathway activation in MGUS, Smoldering Multiple Myeloma, and Multiple Myeloma

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Abstract

Introduction: In the absence of a single unifying genetic event leading to disease manifestation in MM, current efforts are focused on identifying novel therapies based on pathways that promote tumor cell survival. The PI3K/Akt/mTOR, Ras/Raf/MEK/ERK and Jak2/Stat3 are three such pathways, which when blocked has shown promising anti MM responses in pre clinical studies. However, the pattern of signaling pathway activation in asymptomatic precursor states of MGUS and smoldering multiple myeloma (SMM) compared to symptomatic MM has not been characterized. Here, we have examined the activated levels of the above mentioned pathways by immunohistochemical (IHC) analysis across the spectrum of MGUS, SMM, and newly diagnosed MM and assessed for impact on clinical outcomes based on pathway activation.

Methods: Formalin-fixed paraffin embedded bone marrow biopsy samples were obtained during routine clinical care for consenting patients seen at Mayo Clinic. Primary antibodies used were anti-p-Akt (S473), p-mTOR (Ser2448), p-ERK1/2 (Thr202/Tyr204), and p-STAT3 (Tyr705). Staining intensity of plasma cells was scored as: 0, no staining; 1, light staining of <50% of cells; 2, detectable staining in >50% of cells; and 3, dark staining obscuring the majority of tumor cells. Fisher's exact test was used to compare nominal variables and Kaplan Meier analysis was used for time to progression and overall survival analysis.

Results: A total of 21 MGUS, 20 SMM, and 18 MM patients were reviewed. Strong expression of p-Akt (2-3+ IHC score) was present in 15% of MGUS cases versus 62% of SMM and 61% of MM patients (p=0.037). Phospho-mTOR expression was detectable in 93% of patients (n=55), however strong p-mTOR expression was observed in SMM and MM patients compared to MGUS patients (100% and 94% versus 45%; p=0.001). 61% of MM cases exhibited strong p-ERK expression versus 34% in SMM and 15% in MGUS (p=0.042). Higher p-STAT3 expression was observed in MM versus SMM and MGUS (39% strong intensity in MM versus 12% and 10% in SMM and MGUS, respectively; p=0.15). Increased p-mTOR activity correlated with 2-3+ expression of p-Akt (S473) across all disease groups (p=0.023), suggestive of possible mTORC2-mediated Akt activation. Time to progression (TTP) in SMM patients with high versus low p-ERK activity was significantly shorter (76 months vs 16 months; P=0.046).

Conclusions: Comprehensive IHC analysis across the spectrum of plasma cell disorders demonstrated increased activity of multiple key signaling pathways as patients progress from MGUS to SMM and MM. Our results also suggests that up regulation of the PI3K/Akt/mTOR pathway is an early event in pathogenesis when compared to the other two signaling pathways examined.

Objectives

- To evaluate the activation of PI3K/Akt/mTOR, Ras/Raf/MEK/ERK, and Jak2/Stat3 signaling pathways in asymptomatic precuror states of MGUS and smoldering multiple myeloma compared to symptomatic multiple myeloma by immunohistochemistry (IHC) analysis.
- To assess for impact on time to progression based on signaling pathway activation.

Methods

- MGUS, SMM, and newly diagnosed MM patients who consented provided formalin-fixed paraffin embedded (FFPE) bone marrow biopsy samples during routine clinical care.
- MGUS, SMM, and MM cohorts defined by International Myeloma Working Group criteria.
- Baseline clinical characteristics of the three cohorts was collected including overall survival and time to progression (from MGUS or SMM→MM or MM→first progression)
- Staining intensity of plasma cells was scored as: 0, no staining; 1, light staining of <50% of cells; 2, detectable staining in >50% of cells; and 3, dark staining obscuring plasma cells.
- IHC scores were categorized into two groups: Low (0-1+) or High Expression (2-3+).
- Fisher's exact test and Kaplan Meier analysis were used for statistical analysis.

Results

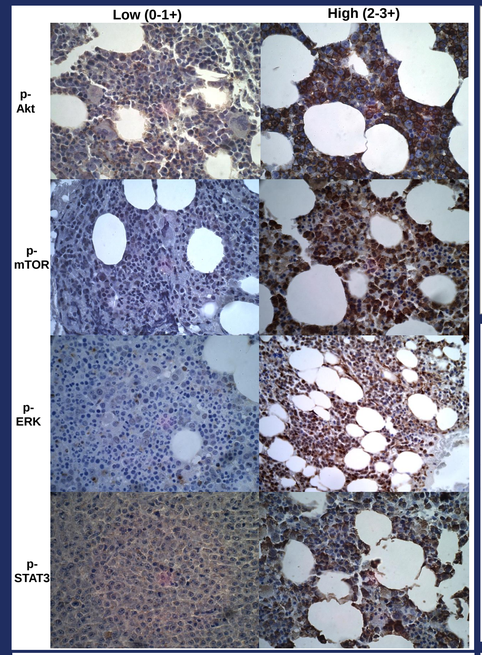

Figure 1. Immunohistochemical expression of signaling proteins phosphorylated Akt (Serine 473), phosphorylated-mTOR, phosphorylated ERK, and phosphorylated STAT3. Representative examples of low expression (0/1+ score) and strong expression (2/3+) are shown.

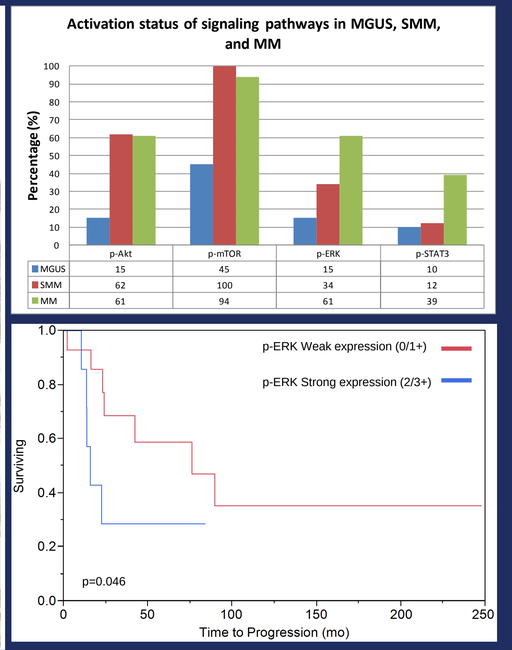

Figure 2. Time to progression (TTP) from SMM to MM requiring treatment based on ERK activation status. Median TTP in strong p-ERK SMM was 16 months versus 76 months in weak p-ERK expression patients (p=0.046). No other significant differences in TTP of overall survival based on pathway activation were observed.

Table 1: Relationships of activated pathways

		p-Akt (Ser473)			p-mTOR			p-ERK			p-STAT3		
		Low	High	P	Low	High	P	Low	High	P	Low	High	P
p-Akt	Low				10 (17%)	22 (37%)	0.023	23 (39%)	9 (15%)	0.19	22 (40%)	7 (12%)	0.42
	High				2 (3%)	25 (42%)		15 (25%)	12 (20%)		22 (40%)	4 (7%)	
p-mTOR	Low	10 (17%)	22 (37%)	0.023				9 (15%)	3 (5%)	0.39	11 (20%)	1 (2%)	0.25
	High	2 (3%)	25 (42%)					29 (49%)	18 (31%)		33 (60%)	10 (18%)	
p-ERK	Low	23 (39%)	15 (25%)	0.19	9 (15%)	29 (49%)	0.39				26 (47%)	8 (15%)	0.41
	High	9 (15%)	12 (20%)		3 (5%)	18 (31%)					18 (32%)	3 (5%)	

Conclusions

1. Increased activity of multiple key signaling pathways is present as disease progression from asymptomatic states of MGUS and SMM to symptomatic MM.
2. PI3K/Akt/mTOR pathway activation in SMM is similar to MM, which suggests upregulation of this pathway may be an early event in pathogenesis.
3. Increased p-mTOR activity correlated with high p-Akt activity across all disease groups, possibly reflective of mTORC2-mediated Akt activation.
4. High pERK activity was associated with significantly shorter time to progression in SMM patients (76 vs 16 months, p=0.046) although analysis is limited by small patient numbers. Further analysis with larger sample sizes of pathway activation as a prognostic marker for SMM is planned.