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Rac/cdc42 inhibitor MBQ-167 in the estrogen receptor positive breast cancer tumor microenvironment

Ailed M. Cruz-Collazo[1], Nicole Zaragoza-Rodriguez[2], Homer Fogle[2], Anamaris Torres-Sánchez[1], Nilmary Grafals[1], Viviana Negrón[3], Victor Carlo[3], Riccardo Papa[4], Stephanie Dorta-Estremera[2,5], Hector Franco[2], Suranganie Dharmawardhane[1,6]

[1]Department of Biochemistry, University of Puerto Rico (UPR) Medical Sciences Campus (MSC); [2]Division of Clinical and Translational Cancer Research, UPR Comprehensive Cancer Center; [3]Auxilio Mutuo Hospital Cancer Center, [4]Department of Biology, UPR Rio Piedras; [5]Department of Microbiology, UPR MSC; [6]MBQ Pharma, Inc., San Juan, PR.

Abstract

MBQ-167 is a dual Rac and Cdc42 inhibitor with significant anti-tumor growth and metastasis blocking activities in metastatic breast cancer cell and mouse models. MBQ-167 reduces both Rac and Cdc42 activation by GTP exchange and consequently, decreases phosphorylation of the common Rac and Cdc42 downstream effector p21-activated kinase (PAK). MBQ-167 is currently in Phase 1 clinical trials in advanced breast cancer patients, and so far, has shown safety and superior bioavailability up to 180 mg/kg BID (NCT06075810). Immune cell phenotyping from mouse tissue (tumor and spleen) and plasma from mice with tumors established from TNBC tumors demonstrated significant changes in myeloid cells and T lymphocytes indicating immune modulatory anticancer effects of MBQ-167 in the TME via inhibition of Rac and Cdc42. Immunohistochemistry (IHC) of breast cancer patient tissues demonstrate that Rac and Cdc42 are expressed in basal, HER2+ and (or) ER/PR+ breast cancers regardless of grade or type. However, the levels of their active downstream effector PAK, identified via IHC using a phospho (P) PAK antibody, is elevated in invasive breast cancer. Since the majority of breast cancers (~70%) are ER+, the objective in this study is to determine the effect of MBQ-167 in ER+ breast cancer, using an ex vivo drug testing platform. ER+ breast cancer patient tissues (>2 cm lesion) were collected immediately following surgical resection. Tissue slices (5 mm) were incubated in 0-1000 nM MBQ-167 for 24 h in ex vivo culture and fixed for H&E staining and IHC for PAK or p-PAK. A set of treated tissue slices were also snap frozen in liquid N2 and subjected to single cell RNA Seq to determine the effect of MBQ-167 on cellular heterogeneity in the TME. Results (N=5) show that when ER+ tissue slices following vehicle or 1000 nM MBQ-167 treatment were fixed and subjected to IHC using antibodies to p-PAKT423 or PAK, p-PAK levels were significantly reduced by ~70% without affecting total PAK levels. From the frozen samples treated with vehicle or 1000 nM MBQ-167 for 24 hrs, a total of 10 libraries was created for single cell RNA seq for 5 ER+ tumors. These data allowed us to systematically annotate the changes in gene expression in response to MBQ-167 in the different cell types within these tumor specimens. In conclusion, MBQ-167 reduces active p-PAK levels in ER+ breast cancer, and p-PAK levels can be used to demonstrate target engagement for the Rac/Cdc42 inhibitor MBQ-167 in ER+ breast cancer. These results inform the dynamics of Rac/Cdc42 inhibitor therapy in breast cancer patients and forward Phase 2 trials for this novel therapeutic in all breast cancer patients regardless of the clinical subtype.

Introduction

Breast cancer can be cured if detected early; however, when breast cancer metastasizes, survival rates are reduced from 90% to 20%. Dysregulation of Rho GTPases (Rho, Rac, Cdc42), which are critical for cancer cell migration, are central to metastatic cancer progression. Therefore, we focus on developing and characterizing Rac/Cdc42 inhibitors in breast cancer. Rac and Cdc42, when activated by cell surface receptors, regulate multiple pro-cancer signal cascades including those activated by their direct downstream effector p21-activated kinase (PAK)[1]. In addition, Rac and Cdc42 are pivotal cytoskeletal and signaling regulators during both innate and adaptive immune cell migration relevant for immune suppression in the tumor microenvironment (TME) and are essential for macrophage and neutrophil recruitment to the TME. Therefore, Rac and Cdc42 serve as ideal molecular targets that regulate both cancer cell and immune cell migration, as well as their crosstalk signaling at the TME. To test the **hypothesis** that Rac/Cdc42 inhibition negatively affects both cancer cells and immune and stromal cells in the TME, we analyzed the effects of MBQ-167, a Rac/Cdc42 inhibitor that we have developed[2-4], on the transcriptome of cancer and associated cells in breast cancer patient tissues. Estrogen receptor (ER) positive invasive breast cancer tissues were selected for this study, because the current participants in the Phase 1 clinical trial have ER+ advanced breast cancer. So far, MBQ-167 has no adverse effects in the participants tested with up to 180 mg/kg MBQ-167 BID for 21 days. This companion study demonstrates the validity of treating ER + breast cancer with MBQ-167 by testing its e efficacy in ex vivo cultures of ER+ breast cancer patient tissue. The single cell RNA seq analysis also identifies the genes and signaling pathways dysregulated by MBQ-167 treatment in both cancer and stromal cells in the TME.

Table 1: Patients profiles. Patient tissue was received from Hospital Auxilio Mutuo, San Juan, PR, immediately following surgery, under human IRB protocol no. 2406245359R001.

Patient ID	S3	S4	S5	S6	S7
Pathology info	ER (+), PR (+), Her2 (-); Stage 2; partial masectomy; 2 rounds chemotherapy	ER (+), PR (-), Her2 (-); Invasive ductal carcinoma; Grade 3; 2.6 cm; KI-67: 60%; T2N0M0; may have got some amiridex but no upfront chemo	ER (+), PR (-), Her2 (-); Invasive lobular carcinoma	ER (+), PR (-), Her2 (-); aparently encapsulated papillary carcinoma; Stage 1; Ki67 < 10%; Hepatitis C.	ER (+), PR (+), Her2 (-); Invasive lobular carcinoma Grade 2; T1 (by sono 2 cm), T2 (by MRI 3.3 cm); KI67 <10%

Methodology

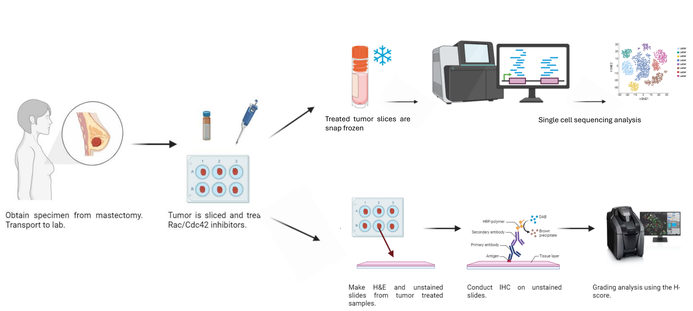
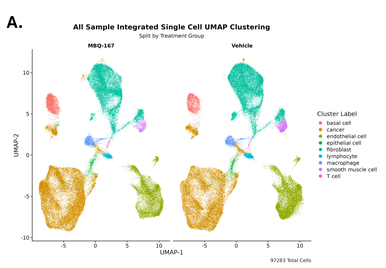


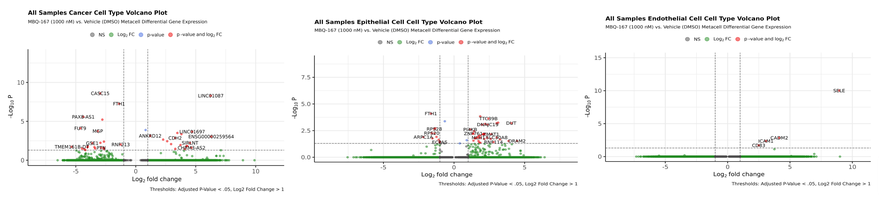
Figure 1 . Breast cancer tissue processing and drug treatment. Tumor tissue in mammary epithelial cell media were sliced into 500mm thick sections using a vibratome and incubated for 4 hrs at a 37^0 CO_2 incubator while shaking. Tissue slices were treated with vehicle or 1000nM MBQ-167 for 24 hrs. Tissues were divided and a portion snap frozen for single cell sequencing, and a portion fixed in 10% formaldehyde and prepared for immunohistochemistry (IHC).

Single cell sequencing

Figure 2. Single cell sequencing data. **A.** Umap cluster of all cell types found in the tissue samples. **B.** Volcano plot for the genetic profile of all the cancer, epithelial and endothelial cells in the tissue samples. **C.** Single cells genes regulation in the tissue samples.



B.



C.

Top 25 downregulated pro-cancer protein coding genes in cancer cells

Gene	Function	Adj. P	Log2 FC
FUT9	Fucosyltransferase 9, aberrant glycosylation leads to cancer	3.8 E-09	-4.63
PTCHD4	Patched domain containing 4, p53 responsive gene	9.9 E-10	-3.45
MGP	Matrix gla protein, poor prognosis in multiple cancers	8.7 E-09	-3.17
PCBP3	Poly C binding protein, pancreatic, breast, brain cancer	1.1 E-08	-2.8
UNC5C	Netrin receptor, neural development	6.9 E-12	-2.14
HS3ST2	Heparin sulfate glucosoamine 3-sulfotransferase2	4.9 E-07	-2.11
ADAMTSL1	Punctin, metalloproteinase implicated in breast cancer	1.0 E-07	-2.09
TRPA1	Ion channel, transient receptor potential Ankrun 1, breast cancer	1.1 E-08	-1.8
H2BC8	H2B clustered Histone 8, prostate cancer biomarker	5.3 E-07	-1.77
GABRE	Gamma amino butyric acid type A receptor subunit	1.9 E-06	-1.75
SLC4A5	Sodium bicarbonate cotransporter, induced by hypoxia	3.6 E-07	-1.63
PCSK1	Proprotein convertase, prohormones to active hormones	1.3 E-08	-1.69
KCNJ3	Kir 3.1 potassium channel, overexpressed in breast cancer	3.5 E-07	-1.63
HMOX1	Hene oxygenase 1, defense against oxygen stress	2.0 E-07	-1.51
HS3ST3A1	Heparan sulfate glucosoamine 3-sulfotransferase 3A1	1.2 E-06	-1.44
CDK18	Cyclin dependent kinase 18, breast cancer progression	8.6 E-07	-1.43
MT1X	Metallothienein 1X protects against oxidative stress	2.3 E-11	-1.41
MT-ND4L	NADH dehydrogenase 4, subunit of complex 1	7.4 E-07	-1.34
GRIA2	Subunit of AMPA glutamate receptor	4.1 E-09	-1.31
ZNF423	Zn finger transcription factor, brain development, neuroblastoma	2.4 E-07	-1.3
KIRREL3	Kin of irregular chiasm-like protein3, nephrin family, glioma	5.2 E-07	-1.17
SPATS2L	Spermatogenesis associated serine rich2 like, RNA binding	2.1 E-07	-1.17
PLCG2	Phospholipase C gamma2, cancer and immunological disorders	2.5 E-07	-1.12
RPL36	Ribosomal protein L36	1.5 E-07	-1.11
ECPAS	ECM29 proteosome adaptor and scaffold, cell survival	3.8 E-07	-1.1

Top seven upregulated tumor suppresors in cancer cells

Gene	Function	Adj. P	Log2 FC
MKRN3	Makorin ring finger protein3, E3 ligase, acs as tumor suppressor by ubiquitinating PABPC1	2.6 E-10	2.53
BCHE	Butyrylcholinesterase, drug detoxification	2.1 E-10	2.42
CUX2	Cut-like Homeobox2, transcription factor in brain development	3.7 E-09	2.14
ADAMTS9	A disintegrin and metalloproteinase with thrombospondin motifs	7.6 E-14	1.35
ZMYND8	Zn finger MYND-type containing 8, transcription and chromatin remodeling	1.8 E-09	1.07
ITIH5	Inter alpha trypsin inhibitor heavy chain 5	0.018	1
TUSC3	Tumor suppressor candidate 3, Mg uptake and N-linked glycosylation	1.0 E-05	1.47
Non TP			
POTEE	POTE Ankyrin domain family member E	2.5 E-08	3.33
POTEJ	POTE Ankyrin domain family member J	2.6 E-07	3.55
SELE	E selectin, neutrophil adhesion	2.6 E-07	3.8

Top downregulated pro-cancer protein coding genes in endothelial cells

Gene	Function	Adj. P	Log2 FC
PLCXD3	Phosphatidyl specific phospholipase CX domain containing 3, cancer progression	0.017	-1.12
POSTN	Periostin, tissue repair, increased metastasis and chemoresistance	0.002	-0.97

Top downregulated pro-cancer protein coding genes in fibroblasts

Gene	Function	Adj. P	Log2 FC
KCTD16	Potassium Channel Tetramerization Domain Containing 16	0.04	-1.79
TBX5	T box transcription factor 5	0.04	-1.54
LRRC6	Leucine Rich Repeat Containing 66	0.05	-1.42
EDA2R	ectodisplasin A receptor, ectodermal tissue development	0.05	-1.01
GPR176	G protein coupled receptor 76	3.21E-05	-0.97

H&E and Immunohistochemistry

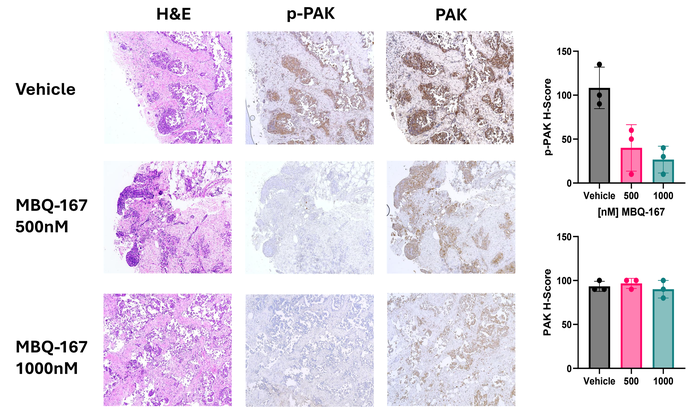


Figure 3. ER+ breast cancer patient tissue respond to MBQ-167 treatment by reducing p-PAK levels without affecting PAK expression.

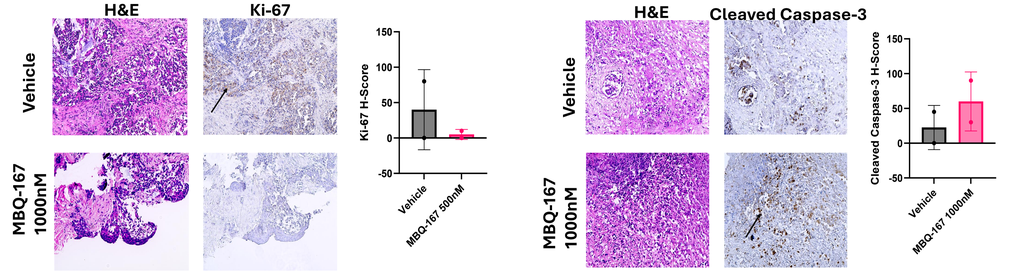


Figure 4. MBQ-167 also reduces cell proliferation, as evidenced by KI-67 staining and increases apoptosis as evidenced by Cleaved caspase3 staining.

Conclusions

- **ER+ breast cancers respond to the Rac/Cdc42 inhibitor MBQ-167 by demonstrating reduced activity as quantified by using the phosphorylation status of their downstream effector PAK.**
- **Cancer cells, epithelial cells, endothelial cells, fibroblasts, macrophages, and basal cells in the TME respond to MBQ-167 by a down regulation in pro-cancer genes and an upregulation in tumor suppressor genes.**
- **This study demonstrates the efficacy of MBQ-167 in ER + breast cancer patients and validates its use for advanced ER+ breast cancer therapy.**

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