3006

Proteomic Profiling of a Novel Anti-Cancer Small Molecule Inhibitor of Sec61

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BACKGROUND

- Secreted and transmembrane (TM) proteins play key roles in malignant transformation and tumor growth. The majority of those proteins require translocation through Sec61 translocon into the ER for further processing (Figure 1).
- Sec61 represents a unique therapeutic target for cancer treatment through blockade of protein secretion.
- We initiated a drug discovery programs to identify novel inhibitors of Sec61 with increased selectivity and tolerability for treatment of malignant diseases.
- KZR-8834, a top candidate identified through a medicinal chemistry campaign, induced cell death in multiple cell lines in vitro with rapid activation (≤8 hours) of caspase 3/7 via dual activation of caspases 8 and 9. Weekly administration of KZR-8834 was effective in multiple xenograft models in which >90% tumor growth inhibition could be achieved without significant body weight loss or clinical signs of toxicity.
- We utilized quantitative proteomic methods to study KZR-8834 for inhibition of protein secretion and global modulation of protein homeostasis in sensitive and resistant tumor cell lines.

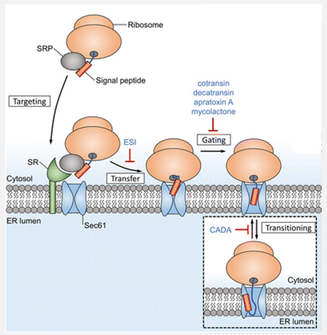


Figure 1. Co-translational Translocation in Eukaryotes through Sec61 Translocon

Figure 2. Myeloma (MM) Cell Lines Show Different Sensitivity to KZR-8834 Treatment *in vitro*

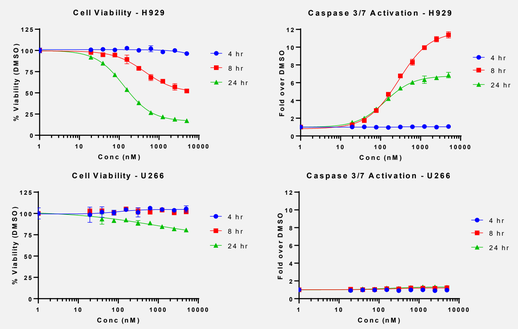


Figure 3. *In vitro* Cell Line Panel Screen of KZR-8834 Reveals Broad Anti-Tumor Activity

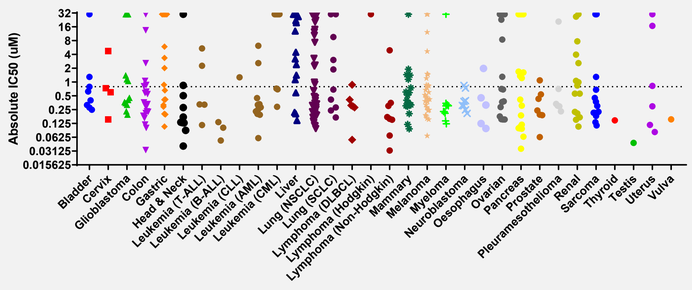
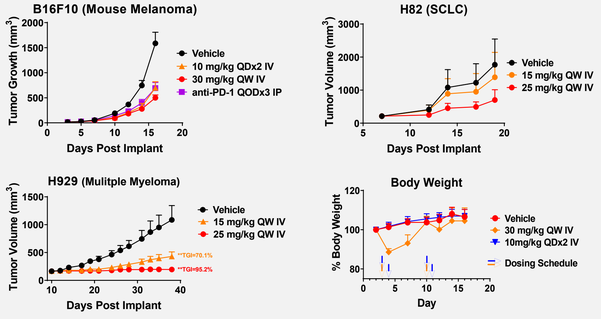


Figure 4. KZR-8834 Exhibits *In-Vivo* Anti-Tumor Activity Against Multiple Tumor Types



MATERIALS & METHODS

- ***Material***: H929 and U266 cells are cultured and treated in-house; all compounds used are synthesized in-house;
- ***Subcellular Fractionation***: After compound treatment, cells are washed twice with PBS and Cytosol Fraction (F1) and Membrane/Organelle Fraction (F2) are obtained using Calbiochem® ProteoExtract® Subcellular Proteome Extraction Kit.
- ***High pH Fractionation***: Digested peptides are fractionated off-line in a high pH reversed-phase mode using spin-columns packed with polymer-based hydrophobic resin and bench top micro centrifugation. Absolute peptide quantities are determined using the quantitative peptide assays for individual fractions before LC-MS sample injection and analysis.
- ***Liquid Chromatography***: Liquid chromatography is performed using Thermo Scientific™ EASY-nLC™ 1200 System, utilizing a 2cm trap column and 50cm C18 Thermo Scientific™ EASY-Spray™ Column heated at 60°C with three-hour gradients.
- ***Mass Spectrometry***: All samples are analyzed on a Thermo Scientific™ Q Exactive Plus mass spectrometer. For SILAC samples, MS-level scans are performed with resolution set to 70,000; AGC Target 3e6; maximum injection time 30 ms; intensity threshold 3.3e4; dynamic exclusion 10 sec. Data dependent MS2 selection is performed in Top Speed mode with HCD collision energy set to 27%, AGC target 2e5 and maximum injection time 60 ms. For TMT-labeled samples, MS-level scans are performed with resolution set to 70,000; AGC target 3e6; maximum injection time 50 ms; intensity threshold 2e4; dynamic exclusion 30 sec. Data-dependent MS2 selection is performed in Top Speed mode with HCD collision energy set to 32%, AGC target 1e5 and maximum injection time 100 ms.
- ***Data Analysis***: Raw data files are analyzed using the SEQUEST® HT search engine in Thermo Scientific™ Proteome Discoverer™ 2.2. Data are searched against the UniProt human protein database and results are filtered using a 1% protein FDR threshold. Further pathway analysis is performed using Qiagen Ingenuity®Pathway Analysis (IPA®) software.

RESULTS

Table 1. Summary of MM SILAC Studies with Different Treatment of KZR-8834

Cell Line	KZR-8834 Treatment	Biological Replicates	Total Protein Quantified		Sec61 Clients Quantified	Sec61 Clients	
						Down 2-fold	Up 2-fold
H929	250nM 6Hrs	Forward LabelingX3 + Reverse LabelingX3	F1	2431	53	2	11
			F2	3228	373	10	21
	250nM 24Hrs		F1	2264	40	2	15
			F2	3328	384	50	35
	1uM 24Hrs		F1	2974	109	9	69
			F2	3664	478	93	58
U266	1uM 24Hrs		F1	2462	65	5	9
			F2	3281	445	60	32

Figure 5. H929 Sec61 Clients Show Dose-dependent Inhibition upon KZR-8834 Treatment (*R*=KZR-8834/DMSO)

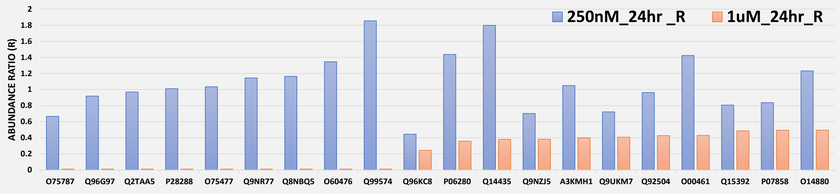


Figure 6. H929 Sec61 Clients Show Time-dependent Inhibition upon KZR-8834 Treatment (*R*=KZR-8834/DMSO)

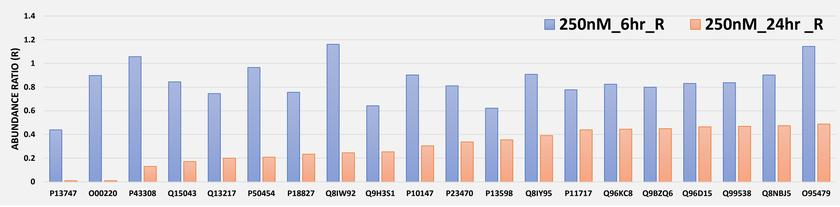


Figure 7. IPA® Pathway Analysis Suggests that Activating Transcription Factor 4 (ATF4) is Activated in H929 Cell Line with KZR-8834 Treatment

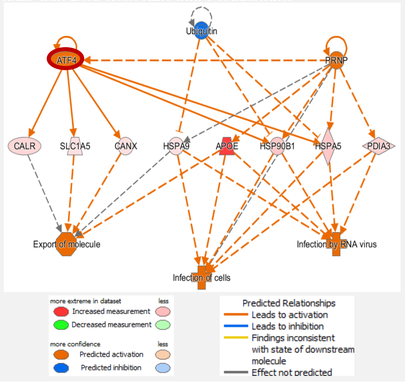
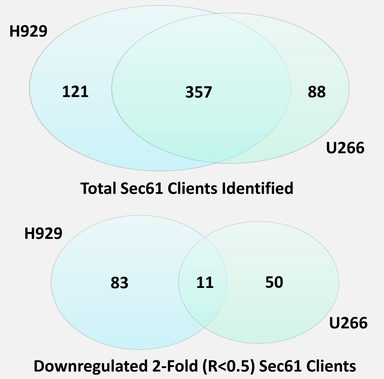


Figure 8. H929 and U266 Cell Lines Exhibit Different Sec61 Clientome that is affected by KZR-8834 Treatment



RESULTS

Table 2. Interesting Targets to Follow Up as Potential Biomarker

Accession	Description	Sec61 Client	Abundance *R**		Main Location
			F1	F2	
P11021	78 kDa Glucose-Regulated Protein (BiP)	Yes	2.59	0.95	ER Lumen
P07237	Protein Disulfide-Isomerase (PDI)	Yes	3.44	0.91	ER Lumen
P14625	Endoplasmin (HSP90B1)	Yes	2.77	1.02	ER Lumen
P27797	Calreticulin (CALR)	Yes	1.90	1.13	ER / Nucleus
P61769	β-2 Microglobulin (B2M)	Yes	N/D	0.62	Membrane
P13598	Intercellular Adhesion Molecule 2 (ICAM2)	Yes	N/D	0.64	Membrane
P10809	60 kDa Heat Shock Protein (HSP60K)	No	2.58	1.17	Mitochondria
P49327	Fatty Acid Synthase (FASN)	No	0.89	0.83	Cytosol
P60709	β-Actin (ACTB)	No	0.99	0.89	Cytosol/Nucleus
P04406	Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH)	No	0.96	0.69	Nucleus/Cytosol

* The abundance ratio *R* is from the TMT study of H929 cells treated with 1uM KZR-8834 for 24 hours.

Figure 9. Western Blot Results of the Targets from Table 2 Support Our Quantitative Proteomics Data

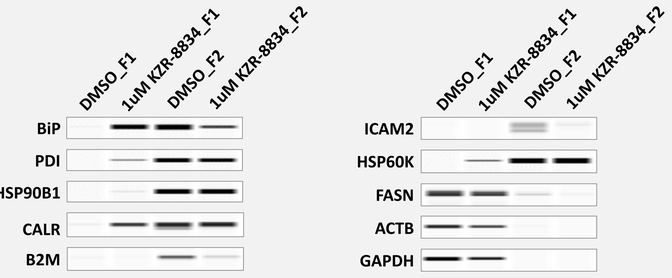
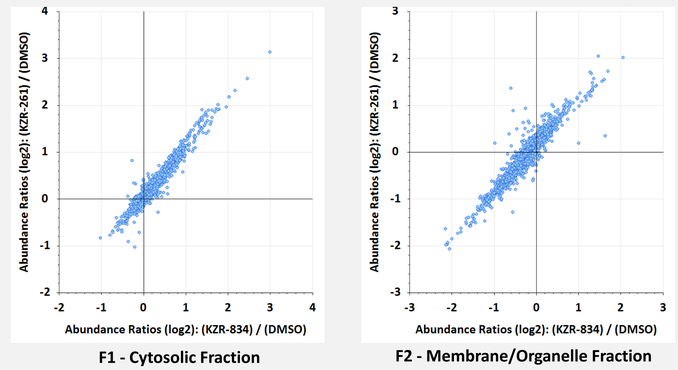


Figure 10. Comparative Proteomics Study Reveals Similar Profiles of KZR-8834 and KZR-9261(analog) in H929



F1 - Cytosolic Fraction

F2 - Membrane/Organelle Fraction

CONCLUSIONS

- Our data suggest that quantitative proteomic profiling is a powerful tool to help us gain a better understanding of KZR-8834 as a potential new therapy for the treatment of malignant diseases.
- Sec61 clients show both time- and dose-dependent inhibition upon KZR-8834 treatment in a sensitive MM cell line.
- A distinct profile of affected Sec61 clientome was noted in a resistant MM cell line comparing to the sensitive MM cell line.
- Activation of ATF4 could be the potential mechanism leading to cell apoptosis in the sensitive MM cell line upon inhibition of Sec61-dependent protein translocation by KZR-8834.
- Quantitative proteomics could also be a powerful tool for compound screening and biomarker discovery *in vitro* and *in vivo*.

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