Supporting Information

Selective inhibition of cancer cells by enzyme induced gain of function of phosphorylated melittin analogues

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**Figure S1.** MelA1-P18 and MelA1-P11 have no ability to reduce membrane lysis potency. Calcein leakage from POPC A) and POPG B) after treatment with MelA1 and MelA1-P11. Calcein leakage from POPC C) and POPG D) after treatment with MelA1 and MelA1-P18. Different doses of peptides were incubated with 25 μM (lipid concentration) calcein-entrapped POPC and POPG vesicles at peptide-to-lipid ratio from 1/3200 to 1/50 for 20 min. The leakage amount achieved platform before 20 min and the calcein fluorescence at platform was used as final intensity, n=3.

**Figure S2.** No apparent change of structure and function after A23T mutation of MelA1. CD spectra of MelA1 and MelA2 interacted with or without POPC vesicles A) and POPG vesicles B). Peptides (10 μM) were incubated with empty POPC and POPG vesicles at peptide-to-lipid ratio of 1:50 for 1.5 h before CD measurement. Calcein leakage from POPC C) and POPG D) after treatment with MelA1 and MelA2. Different doses of peptides were incubated with 25 μM
(lipid concentration) calcein-entrapped POPC and POG vesicles at peptide-to-lipid ratio from 1/3200 to 1/50 for 20 min. The leakage amount achieved platform before 20 min and the calcein fluorescence at platform was used as final intensity, n=3.

Figure S3. Phosphorylation on T23 of MelA2 impairs the α helix conformation and membrane lysis capacity. A) CD spectra of MelA2 and MelA2-P. Peptides (10 μM) were incubated with empty POPG vesicles at peptide-to-lipid ratio of 1:50 for 1.5 h before CD measurements. B) The percentage of α helix conformation in MelA2 and MelA2-P. The secondary structures of peptides were measured by CD and the percentages of α helix were quantified by CDNN software. Calcein leakage from POPC (25 μM) C), POPG (25 μM) D), POPG (100 μM) E) after treatment with MelA2-P and MelA2. Different doses of peptides were incubated with 25 μM or 100 μM (lipid concentration) calcein trapped vesicles at peptide-to-lipid ratio from 1/3200 to 1/50 for 20 min. The leakage amount achieved platform before 20 min and the calcein fluorescence at platform was used as the final intensity, n=3. F)-H) TEM of native POPG, POPG treated with MelA2-P and MelA2. For TEM, peptides (10 μM) were incubated with empty POPG vesicles at peptide-to-lipid ratio of 1:50 for 10 h. The scale bar in TEM images is 500 nm.
Figure S4. Dephosphorylation of MelA2-P by ALP gain peptide membrane lysis potency and cytotoxicity. A) Calcein leakage of POPG vesicles after treatment with active ALP catalyzed MelA2-P and inactive ALP catalyzed MelA2-P. The inactive ALP was derived from 100°C heating of active ALP for 30 min, and every sample had the same amount of ALP protein to avoid the influence of ALP on calcein leakage. The leakage intensity of 100 μM (lipid concentration) calcein-entrapped vesicles at peptide-to-lipid ratio from 1/800 to 1/50 was measured for 20 min. The leakage amount achieved platform before 20 min and the calcein fluorescence at platform was used as final intensity, n=3. B) MelA2-T23E was less toxic to ALP highly active cell Saos-2 than MelA2-P. MelA2-P and MelA2-T23E were dissolved in DMSO to prepare 2 mM peptides stock solutions. Saos-2 cells were treated by 1 μM MelA2-P or MelA2-T23E for 12 h culture, respectively. The cell viability of Saos-2 cells were measured by MTT assay. n=3,* p < 0.05; ** p < 0.01, *** p<0.001.

Peptide identification

MelA1
Analytical RP-HPLC trace of MelA1 (0-20 min: 20%-80% B, 20-40 min: 80-100% B, gradient. A: 100% water +0.06% TFA, B: 20% water +80% acetonitrile +0.06% TFA. All solvents are RP-HPLC grade)

Theoretical molecular weight: 2631.57. Calculated molecular weight: 2630.8±0.2.

MelA1-P11
Analytical RP-HPLC trace of MelA1-P11 (0-20 min: 20%-80% B, 20-40 min: 80-100% B, gradient. A:100% water +0.06% TFA, B: 20% water +80% acetonitrile +0.06% TFA. All solvents are RP-HPLC grade)

Theoretical molecular weight: 2711.54. Calculated molecular weight: 2711.0±0.2.

MelA1-P18
Analytical RP-HPLC trace of MelA1-P18 (0-20 min: 20%-80% B, 20-40 min: 80-100% B, gradient. A:100% water +0.06% TFA, B: 20% water +80% acetonitrile +0.06% TFA. All solvents are RP-HPLC grade)

Theoretical molecular weight: 2711.54. Calculated molecular weight: 2711.3±0.8.

MelA2
Analytical RP-HPLC trace of MelA2 (0-20 min: 20%-80% B, 20-40 min: 80-100% B, gradient. A:100% water +0.06% TFA, B: 20% water +80% acetonitrile +0.06% TFA. All solvents are RP-HPLC grade)

Theoretical molecular weight: 2661.58. Calculated molecular weight: 2661.5±0.2.

MelA2-P
Analytical RP-HPLC trace of MelA2-P (0-20 min: 20%-80% B, 20-40 min: 80-100% B, gradient. A: 100% water +0.06% TFA, B: 20% water +80% acetonitrile +0.06% TFA. All solvents are RP-HPLC grade)

Theoretical molecular weight: 2741.55. Calculated molecular weight: 2741.3±0.1.

MelA2-T23E

Chemical Formula: C_{126}H_{214}N_{32}O_{32}
Exact Mass: 2897.81
Molecular Weight: 2897.24
Analytical RP-HPLC trace of MelA2-P (0-10 min: 20%-80% B, 10-30 min: 80-100% B, gradient. A:100% water +0.06% TFA, B: 20% water +80% acetonitrile +0.06% TFA. All solvents are RP-HPLC grade)

Theoretical molecular weight: 2689.24. Calculated molecular weight: 2688.5±0.1.