New approach to molecular drug design yields highly promising bladder cancer drug candidate

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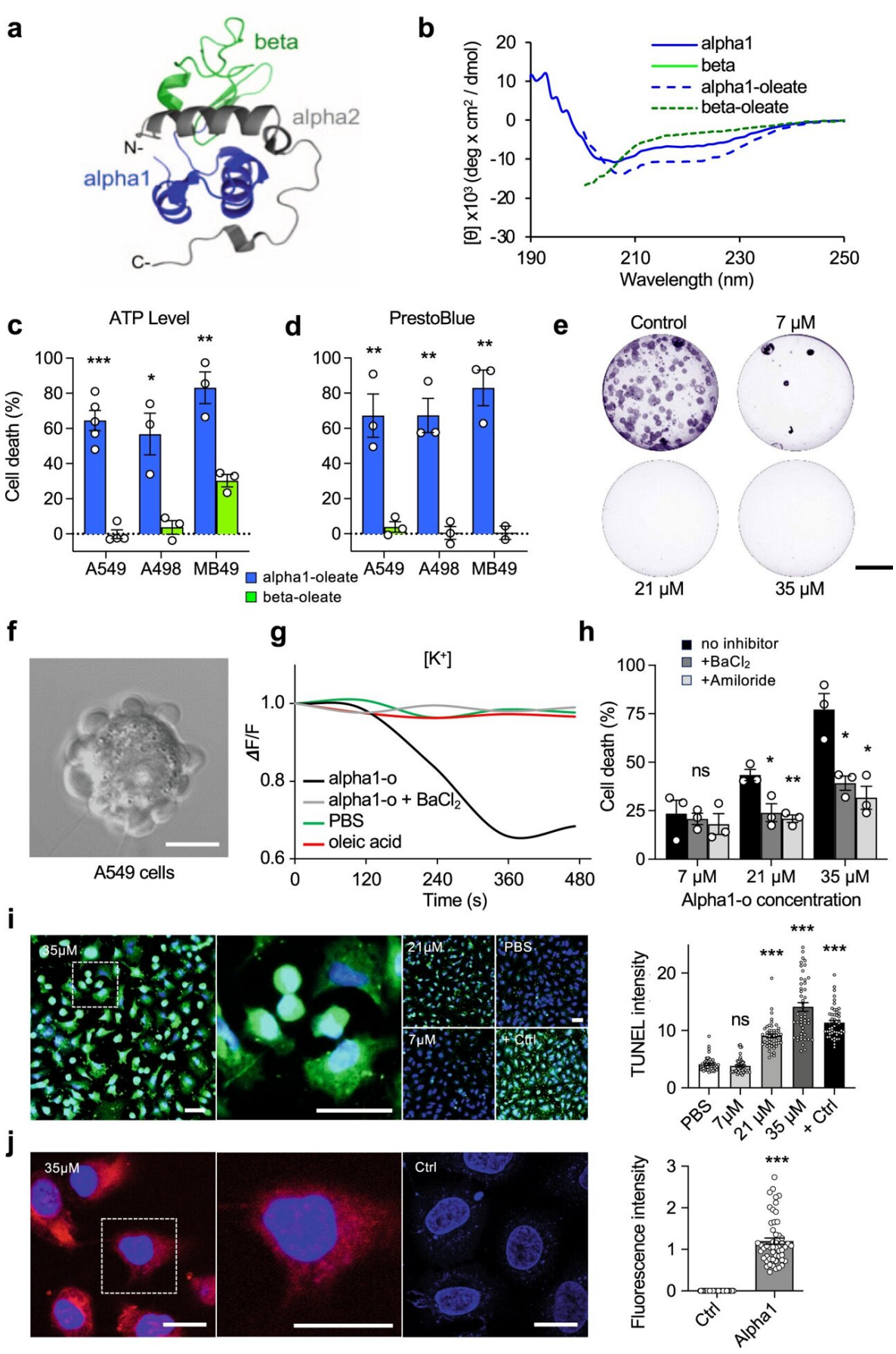
a
beta
alpha2
N-
alpha1
C-
b
alpha1
beta
alpha1-oleate
beta-oleate
[θ] x10³ (deg x cm² / dmol)
Wavelength (nm)
c
ATP Level
Cell death (%)
A549
A498
MB49
alpha1-oleate
beta-oleate
d
PrestoBlue
e
Control
7 µM
21 µM
35 µM
f
A549 cells
g
[K⁺]
ΔF/F
alpha1-o
alpha1-o + BaCl2
PBS
oleic acid
Time (s)
h
no inhibitor
+BaCl2
+Amiloride
Cell death (%)
Alpha1-o concentration
i
35µM
21µM
PBS
7µM
+ Ctrl
TUNEL intensity
j
35µM
Ctrl
Fluorescence intensity
Alpha1

Fig. 1: Tumoricidal activity of two non-homologous alpha-helical peptide–oleate complexes. a Ribbon representation of the crystallographically determined three-dimensional structure of human α-lactalbumin (PDB ID: 1B9O), indicating the alpha1 (blue), beta (green), and alpha2 (gray) domains. The calcium ion is not shown. b Far-UV circular dichroism spectra of synthetic alpha1 peptide, beta peptide, and their respective peptide–oleate complexes. c, d Death response in human lung (A549), kidney (A498), and murine bladder (MB49) carcinoma cells, quantified as a reduction in ATP levels (c, P = 3.26E−5 for A549, 0.013 for A498 and 0.005 for MB49, alpha1–oleate compared to beta–oleate) or PrestoBlue fluorescence (d, P = 0.007 for A549, 0.003 for A498 and 0.002 for MB49, alpha1–oleate compared to beta–oleate). Cells were treated with the alpha1–oleate complex (blue) or the beta–oleate complex (green), (3 h, 35 μM, cell death compared to PBS controls). For controls exposed to the naked peptides or oleate alone, see Supplementary Fig. 1d. e Colony assay showing dose-dependent long-term effects of alpha1-oleate. A representative image is shown from two independent experiments. Scale bar = 5 mm. f Alpha1–oleate triggers rapid membrane blebbing in A549 lung carcinoma cells (35 μM, 10 min). Scale bar = 10 μm. A representative image is shown from three independent experiments. g K+ efflux in A549 lung carcinoma cells exposed to alpha1–oleate and inhibition with BaCl2. h Inhibition of cell death by the ion flux inhibitors Amiloride and BaCl2 (100 μM), measured by PrestoBlue fluorescence (P = 0.031 for 21 μM + BaCl2, 0.005 for 21 μM + Amiloride, 0.028 for 35 μM + BaCl2, and 0.014 for 35 μM + Amiloride, compared to no inhibitor). i DNA strand breaks detected by TUNEL staining in alpha1–oleate-treated A549 lung carcinoma cells (n = 50 cells per group). Scale bar = 20 μm. j AlexaFluor568-labeled alpha1–oleate (red) is internalized by A549 lung carcinoma cells. Nuclei are counterstained with DAPI (blue) (n = 52 cells per group). Scale bar = 10 μm. Data are presented as mean ± SEM from three independent experiments, *P