



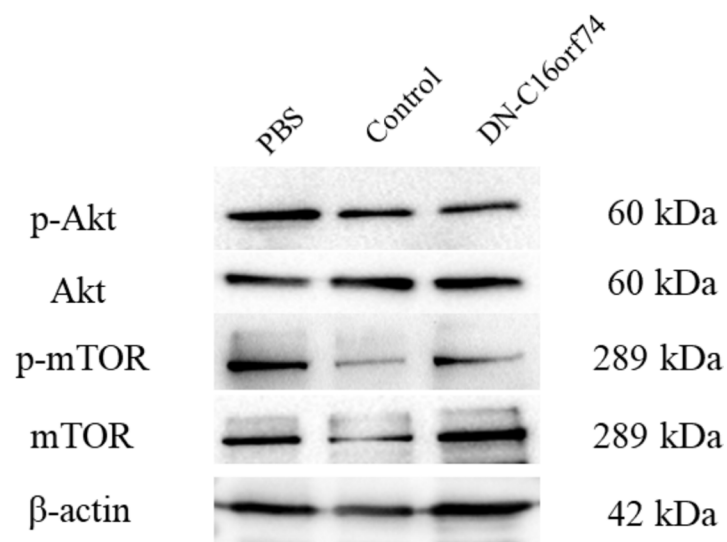
HOKKAIDO UNIVERSITY

Title	Molecular targeting of cell-permeable peptide inhibits pancreatic ductal adenocarcinoma cell proliferation
Author(s)	Sato, Shoki; Nakamura, Toru; Katagiri, Toyomasa et al.
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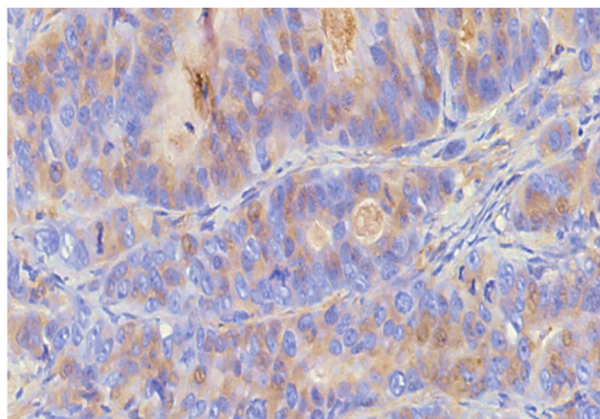


Molecular targeting of cell-permeable peptide inhibits pancreatic ductal adenocarcinoma cell proliferation

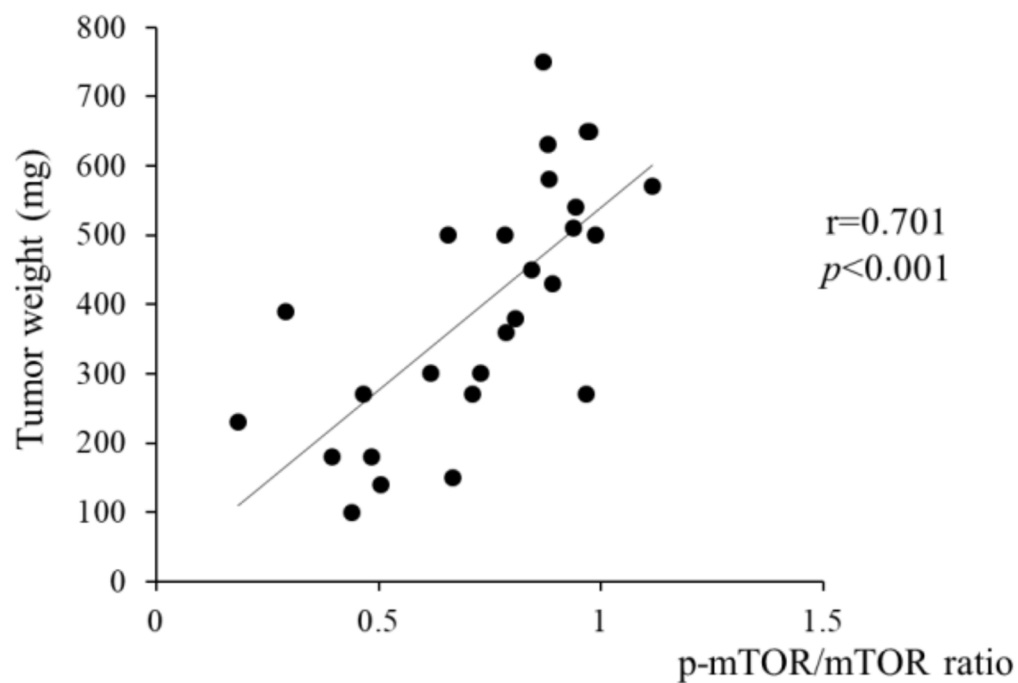
SUPPLEMENTARY MATERIALS



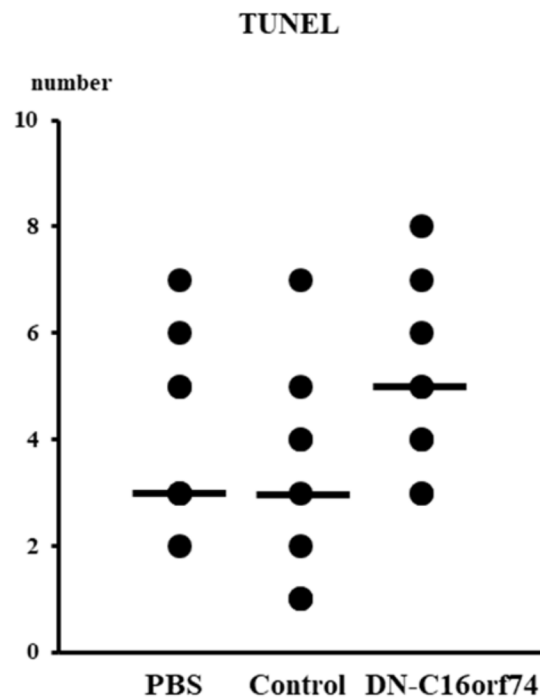
Supplementary Figure 1: To analyze molecular cellular signaling after treatment with DN-C16orf74, western blotting analyses of the expression of Akt, phospho (p)-Akt, mTOR, phospho (p)-mTOR, and β-actin in NHDF cells treated with PBS or peptide were performed. The expression levels of p-Akt and p-mTOR were no change.



Supplementary Figure 2: C16orf74 expression in orthotopic xenograft mouse model tumors (Capan-1 cells) by immunohistochemical staining. Almost all of orthotopic tumor cells had C16orf74 expression.



Supplementary Figure 3: Regression analysis of correlation between orthotopic tumor weight and p-mTOR/mTOR ratio. There was significant correlation between them ($r=0.701$, $p<0.001$).



Supplementary Figure 4: The median TUNEL numbers (range) of the three groups were not significantly different: PBS 3 (2-7) vs. control peptide 3 (1-7) vs. C16orf74 5 (3-8), respectively.

Supplementary Table 1: List of primers for PCR

Gene	Forward	Reverse
<i>C16orf74</i>	5'-GAGGGACTTGGGGAGCAC-3'	5'-TCTGGGTCGATTCTCCATC-3'
<i>GAPDH</i>	5'-GAGAGGCCCTATCCCAACTC-3'	5'-GCGAACTTTATTGATGGTATTCAA-3'

Supplementary Table 2: Lists of antibodies

Western blotting					
Molecule		Clone	Dilution	Manufacture	
Akt	pRb		1:1000	Cell signaling	
Phospho-Akt (Ser473)	mRb	D9E	1:2000	Cell signaling	
mTOR	mRb	D9C2	1:1000	Cell signaling	
Phospho-mTOR (Ser2448)	mRb	7C10	1:1000	Cell signaling	
β-Actin	mMo	C4	1:1000	Millipore	
Immunohistochemistry					
Molecule		Clone	Dilution	Retrieval	Manufacture
Ki67	mMo	MIB-1	1:300	EDTA pH9.0, 20min	Dako
mTOR	mRb	D9C2	1:100	Citric acid pH 6.0, 10min	Cell signaling
Phospho-mTOR (Ser2448)	mRb	49F9	1:100	Citric acid pH 6.0, 10min	Cell signaling

mRb : monoclonal rabbit, mMo : monoclonal mouse