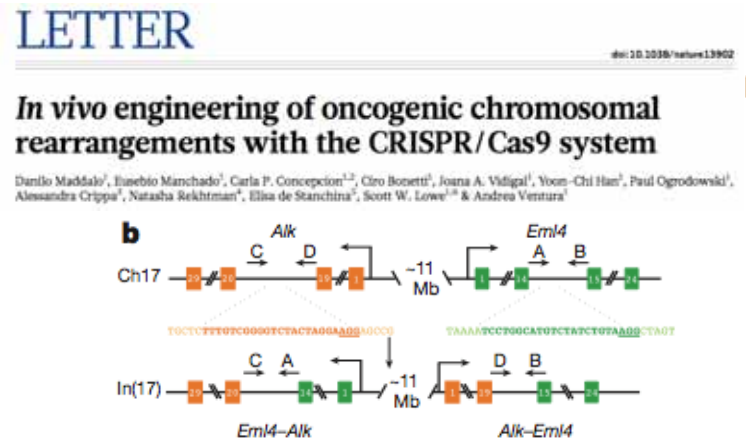
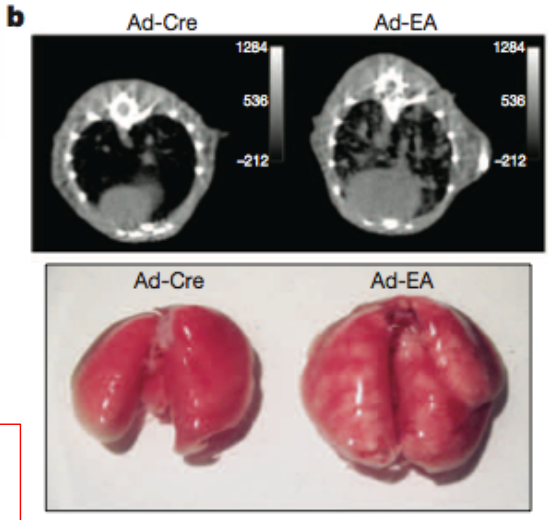
マウスの肺にCRISPRを導入、染色体転座による肺腫瘍を誘導


LETTER

In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system

Danilo Maddalo, Eusebio Manchado, Carla P. Concepcion, Ciro Bonetti, Joana A. Vidigal, Yoon-Chi Han, Paul Ogrodowski, Alessandra Crippa, Natasha Rekhtman, Elisa de Stanchina, Scott W. Lowe & Andrea Ventura



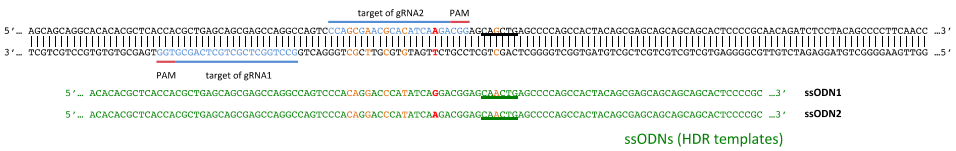
sgRNAとhCas9をアデノウィルスベクターでマウス肺に導入
→ 切断箇所で転座が起こりEml4-Alk融合遺伝子を形成、
腫瘍を引き起こした。

Maddalo et al. *Nature*. 2014 Dec 18;516(7531):423–7.

マウスのゲノムに点変異を導入

Sox9遺伝子の396番目のアミノ酸をリジン(K)からアルギニン(R)に置換

Mouse *Sox9* locus



WT	5′… CAG CGA ACG CAC ATC AAG ACG GAG CAG CTG AGC …3′ … Q R T H I K T E Q L S …
K-to-R mutation via ssODN1	5′… CAC AGG ACC CAT ATC AGG ACG GAG CAA CTG AGC …3′ … Q R T H I R T E Q L S …

Inui et al.. Sci Rep. 2014;4:5396.

骨格や生殖腺に異常が出るか評価

ゲノム編集を用いた研究例

II. 遺伝子修復の試み：

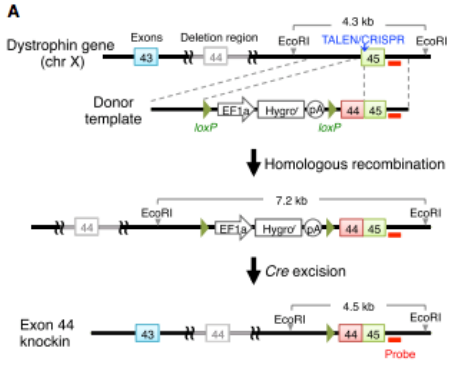
「切断＋挿入＝機能の獲得」を応用し
疾患患者由来の細胞あるいは
疾患モデルマウスの変異を
正しい配列に戻す

筋ジストロフィー患者由来のipsを作成、変異を修復した上で筋肉に分化

Stem Cell Reports
Resource
OPEN ACCESS

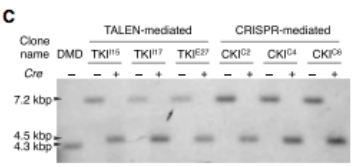
Precise Correction of the Dystrophin Gene in Duchenne Muscular Dystrophy Patient Induced Pluripotent Stem Cells by TALEN and CRISPR-Cas9

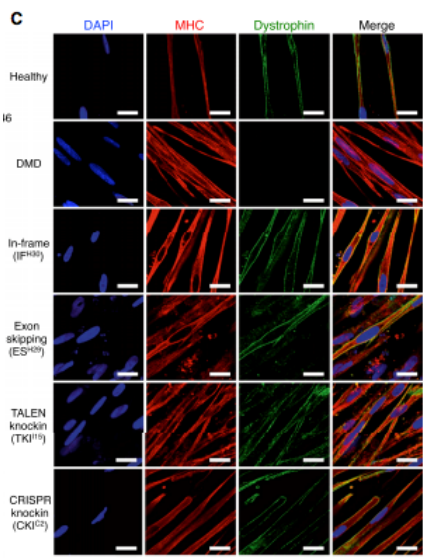
Hongmei Lisa Li,[1] Naoko Fujimoto,[1,2] Noriko Sasakawa,[1] Saya Shirai,[1] Tokiko Ohkame,[1] Tetsushi Sakuma,[4] Michihiro Tanaka,[1] Naoki Amano,[1] Akira Watanabe,[1] Hidetoshi Sakurai,[1] Takashi Yamamoto,[4] Shinya Yamanaka,[1,2,3] and Akitsu Hotta[1,2,3,*]



B

Knockin experiments		Positive ratio by PCR	Single copy ratio by Southern blot	*loxP* excison by *Cre* expression
TALEN-mediated	Golden	95.8% (46/48)	87.5% (7/8)	60.0% (18/30)
	Platinum	89.6% (43/48)	44.4% (4/9)	47.8% (11/23)
CRISPR-mediated	sgRNA1	75% (9/12)	50% (4/8)	65% (23/35)





Li et al. Stem Cell Reports. 2015 Jan 13;4(1):143–54.