

行政院國家科學委員會專題研究計畫 期中進度報告

以 Survivin 基因做為口腔癌分子治療標的之臨床前期試驗 (2/3) 期中進度報告(精簡版)

計畫類別：個別型
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執行期間：95年08月01日至96年07月31日
執行單位：國立臺灣大學醫學院口腔生物科學研究所

計畫主持人：郭彥彬

處理方式：期中報告不提供公開查詢

中華民國 96年05月18日

Survivin是凋亡蛋白質酶抑制劑(inhibitors of apoptosis, IAPs)家族的新成員。它會通過抑制細胞凋亡執行蛋白Caspase-3，來阻礙細胞凋亡。此外，它亦可調節細胞週期，但其機轉目前尚不明瞭。在正常組織中，它只在胚胎某特定時期才會出現，在成人組織則無表現。在大多數轉形的細胞株及癌組織如肺癌、皮膚癌、結腸癌、淋巴癌、胰腺癌、前列腺癌和乳癌中，癌細胞都顯著重新表現了這個蛋白質。Survivin的抗凋亡作用除可使癌細胞得到成長的機會外，且與化療的抗藥性有關。Survivin反義核酸或顯性抑制DNA 被報告能夠誘導肺癌、淋巴癌細胞凋亡和明顯增加化療藥物對這些癌細胞的抑癌作用，並減少實驗動物腫瘤。此外，Bao 等人 (2002) 報告survivin promoter 在癌組織中有專一性的表現。因此，利用 survivin promoter 引入凋亡基因和抑制 survivin 表現的腫瘤療法都有很大的潛力。

在過去我們已完成 62 例口腔上皮變異及96 例口腔癌 survivin免疫染色，其中60例 (97%)上皮變異及94 例(98%)口腔癌為 survivin陽性，但正常口腔上皮組織無表現。

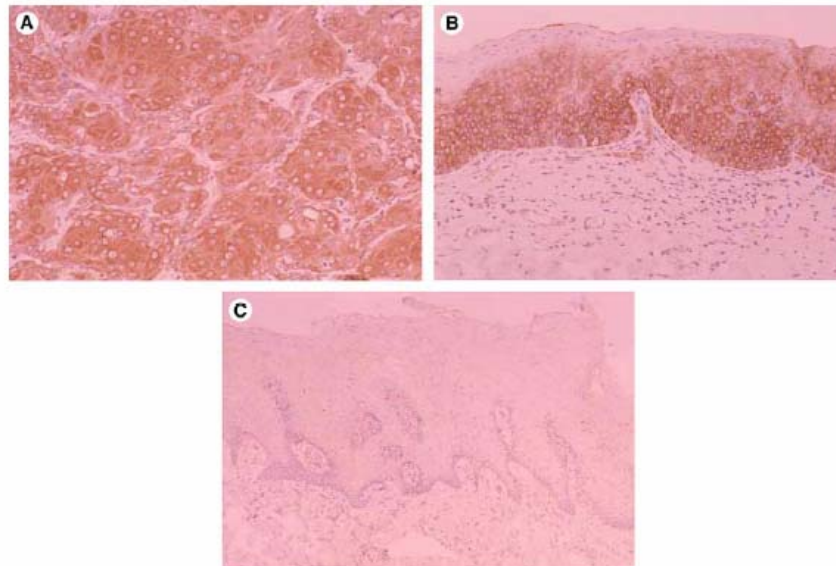


Figure 1 Immunohistochemical staining for survivin. (A) A well-differentiated oral SCC showing positive survivin staining in the cytoplasm of all tumor cells. (B) Moderate epithelial dysplasia demonstrating positive cytoplasmic survivin staining in the spinous epithelial cells. The middle and low spinous cells had stronger cytoplasmic survivin staining than the upper spinous cells. (C) Normal oral mucosa exhibiting negative survivin staining in epithelial cells (A and B, original magnification $\times 50$; C, original magnification $\times 25$).

Survivin於口腔鱗狀細胞癌之活性表現與臨床參數之相關性 則請參閱下表:

	Degree of survivin staining			Mean (SD)	p-Value for LI
	0–25%	26–50%	51–100%		
<i>Age</i>					
≤50	12 (46.2%)	10 (38.5%)	4 (15.4%)	31.6 (19.6)	0.746
>50	13 (36.1%)	19 (52.8%)	4 (11.1%)	32.9 (13.7)	
<i>Sex</i>					
Male	24 (41.4%)	26 (44.8%)	8 (13.8%)	32.2 (16.7)	0.739
Female	1 (25.0%)	3 (75.0%)	0 (%)	35.0 (10.8)	
<i>Location</i>					
Buccal	9 (36.0%)	13 (52.0%)	3 (12.0%)	33.4 (15.5)	0.474
Tongue	10 (50.0%)	8 (40.0%)	2 (10.0%)	28.8 (18.0)	
Others	6 (35.3%)	8 (47.1%)	3 (17.7%)	35.0 (15.7)	
<i>Malignant transformation</i>					
No	25 (47.2%)	22 (41.5%)	6 (11.3%)	30.1 (16.3)	0.008
Yes	0 (0%)	7 (77.8%)	2 (22.2%)	45.6 (8.8)	
<i>Size</i>					
≤1 cm	18 (40.9%)	20 (45.5%)	6 (13.6%)	33.6 (16.4)	0.332
>1 cm	7 (38.9%)	9 (50.0%)	2 (11.1%)	29.2 (16.0)	
<i>Alcohol drinkers</i>					
Yes	15 (34.9%)	20 (46.5%)	8 (18.6%)	35.6 (16.8)	0.017
No	10 (52.6%)	9 (47.4%)	0	25.0 (12.8)	
<i>AQ chewers</i>					
Yes	20 (45.5%)	19 (43.2%)	5 (11.4%)	31.3 (17.4)	0.416
No	5 (27.8%)	10 (55.6%)	3 (16.7%)	35.0 (13.5)	
<i>Smokers</i>					
Yes	23 (41.8%)	24 (43.6%)	8 (14.6%)	32.7 (17.2)	0.603
No	2 (28.6%)	5 (71.4%)	0 (0%)	29.3 (6.1)	

Kaplan-Meier生存分析法分析發現，survivin染色表現高患者其生存期比表現低患者為短。

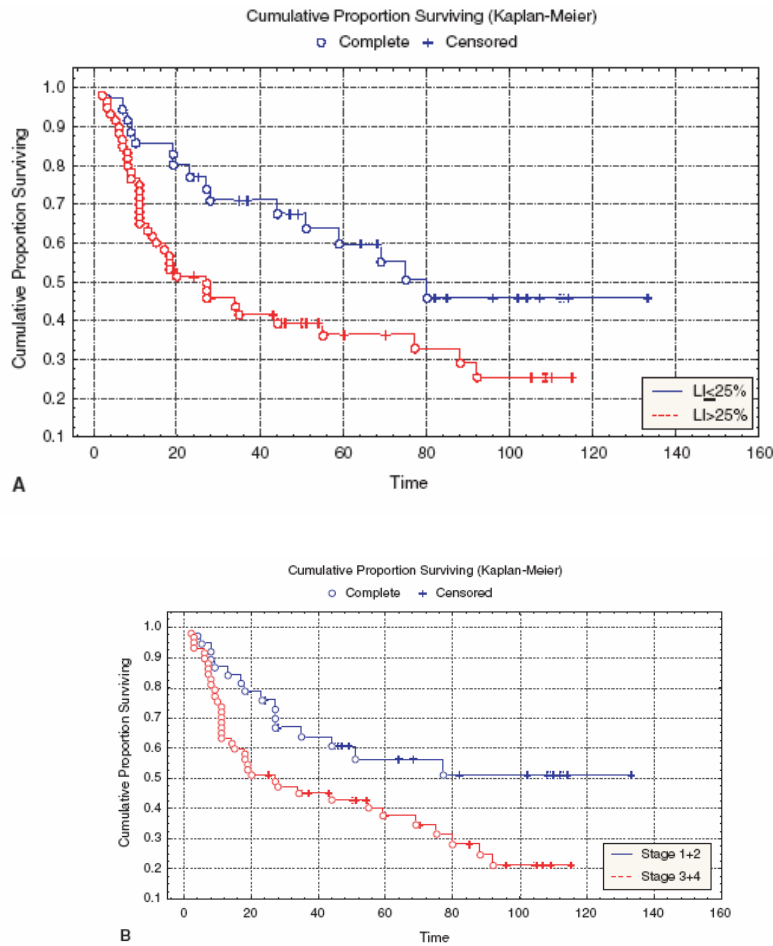


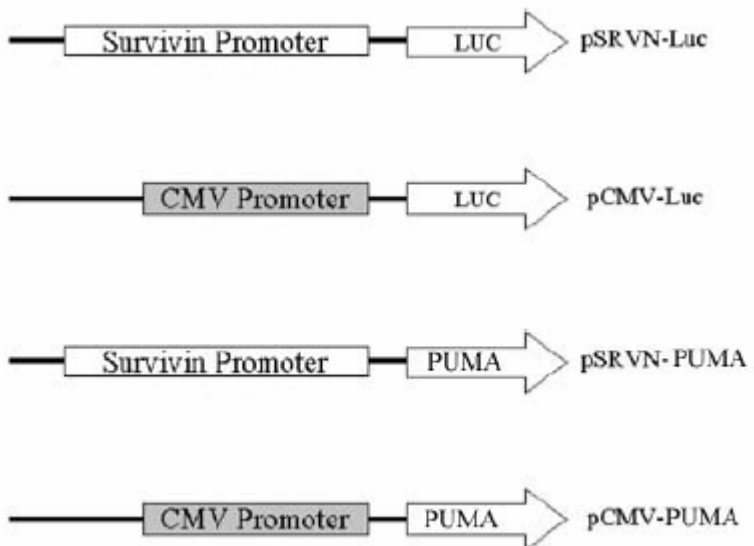
Figure 2 Kaplan–Meier survival curves of 96 patients with oral SCC. (A) Patients with high survivin expression (LI > 25%) tumors had significantly shorter overall survival than those with low survivin expression (LI ≤ 25%) tumors ($p = 0.014$, log-rank test). (B) Patients with advanced stage (S3 + S4) tumors had significantly shorter overall survival than those with less stage (S1 + S2) tumors ($p = 0.012$). (C) Overall survival was significantly shorter in patients with larger tumor size (T3 + T4) than in those with smaller (T1 + T2) tumor size ($p = 0.005$). (D) Overall survival was significantly shorter in patients with positive lymph node metastasis than those without lymph node metastasis ($p = 0.011$). The duration of survival was measured from the beginning of treatment to the time of death (complete) or the last follow-up (censored).

Table 4 Age and multivariable-adjusted relative risks of survivin protein expression and tumor-related factors for mortality among 96 oral SCC patients

Parameters	Age-adjusted model			Age and multivariable-adjusted model		
	Relative risk	95% CI	p -Value	Relative risk	95% CI	p -Value
Age	—	—	—	—	—	—
LI (>25% vs. ≤25%)	1.860	1.047–3.306	0.034	1.639	0.917–2.929	0.096
Stage (per stage)	1.662	1.252–2.205	<0.001	1.615	1.215–2.147	0.001
Tumor (per score)	1.675	1.255–2.236	<0.001	—	—	—
Lymph node metastasis (per score)	1.600	1.184–2.163	0.002	—	—	—

Age and multivariate-adjusted model: p -value < 0.10 as the criteria to select the variables except age.

在過去 10 個月中，我們已經建構好Survivin promoter-Luciferase, Survivin promoter-PUMA 質體。



In Vitro 比較 Survivin promoter-Luciferase 與 CMV promoter-Luciferase 質體表現在各種口腔癌細胞株之間的差異，發現 Survivin promoter 強度與細胞中 Survivin 蛋白的含量呈正相關。平均可以達到 CMV promoter 強度的八成。可見得本計畫可行並可延續下去。

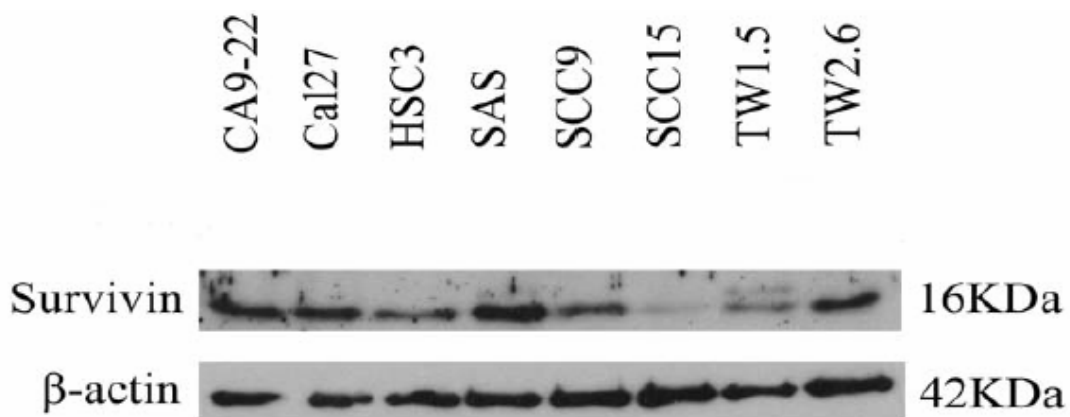
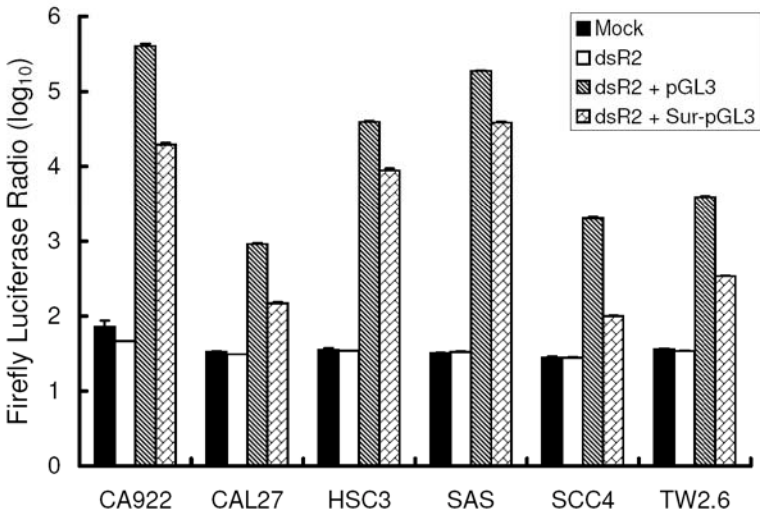
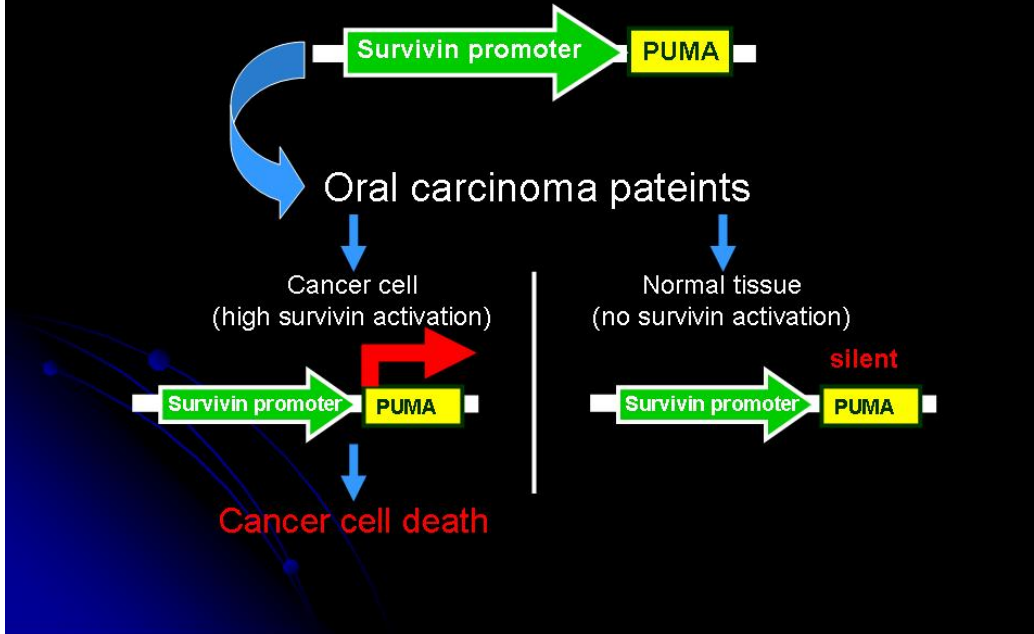


圖. 口腔癌細胞株 survivin 表現量的比較

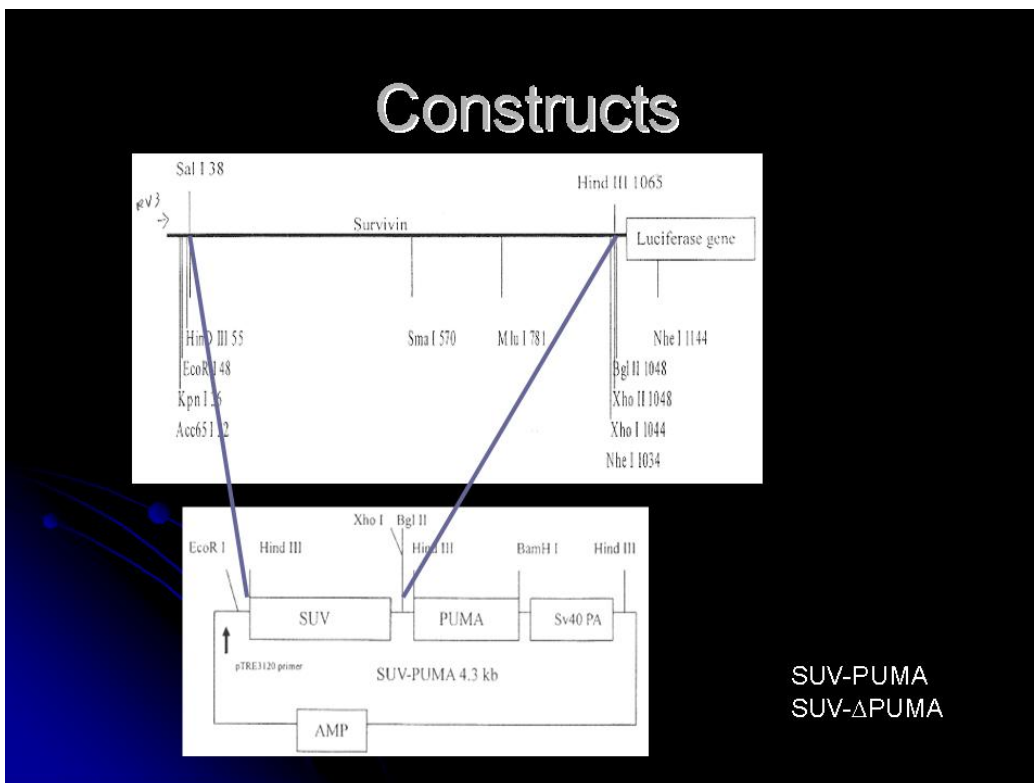
in vitro Analyses of Transgene Expression of The Survivin and CMV Promoters by Transient Transfection

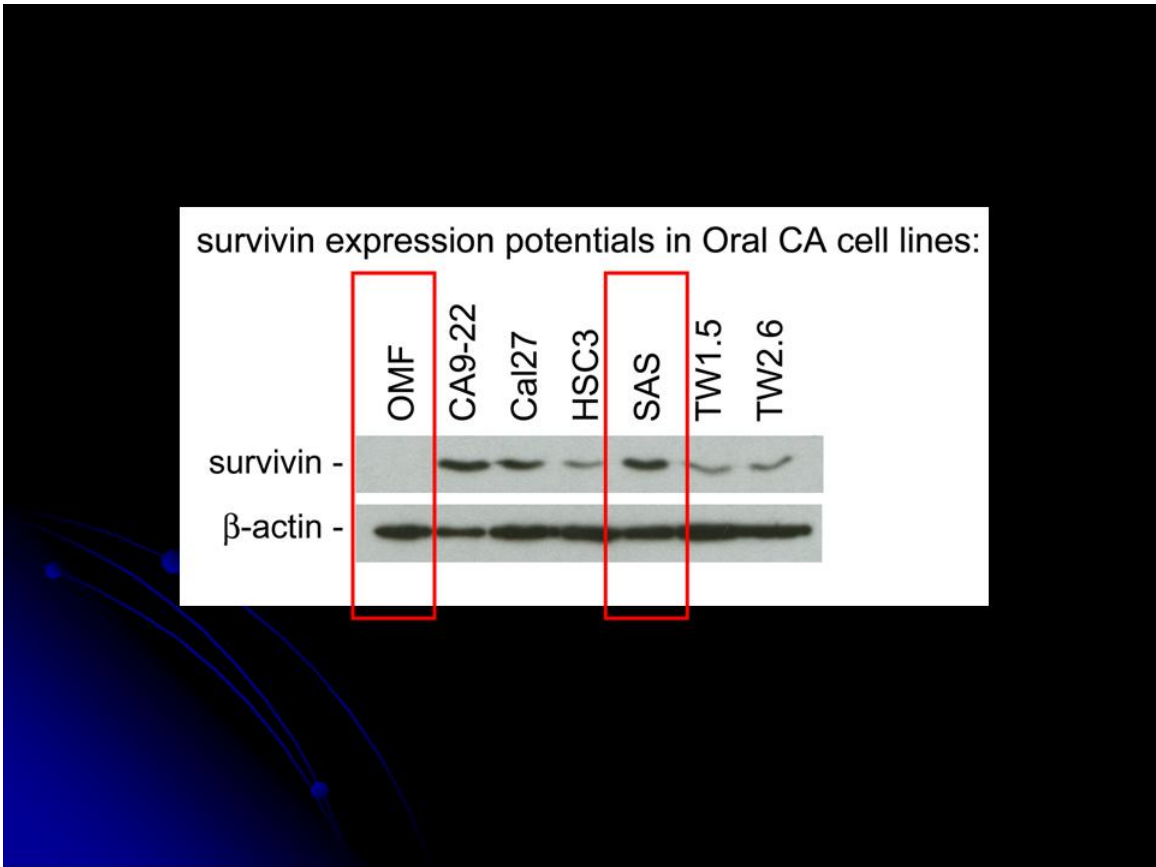
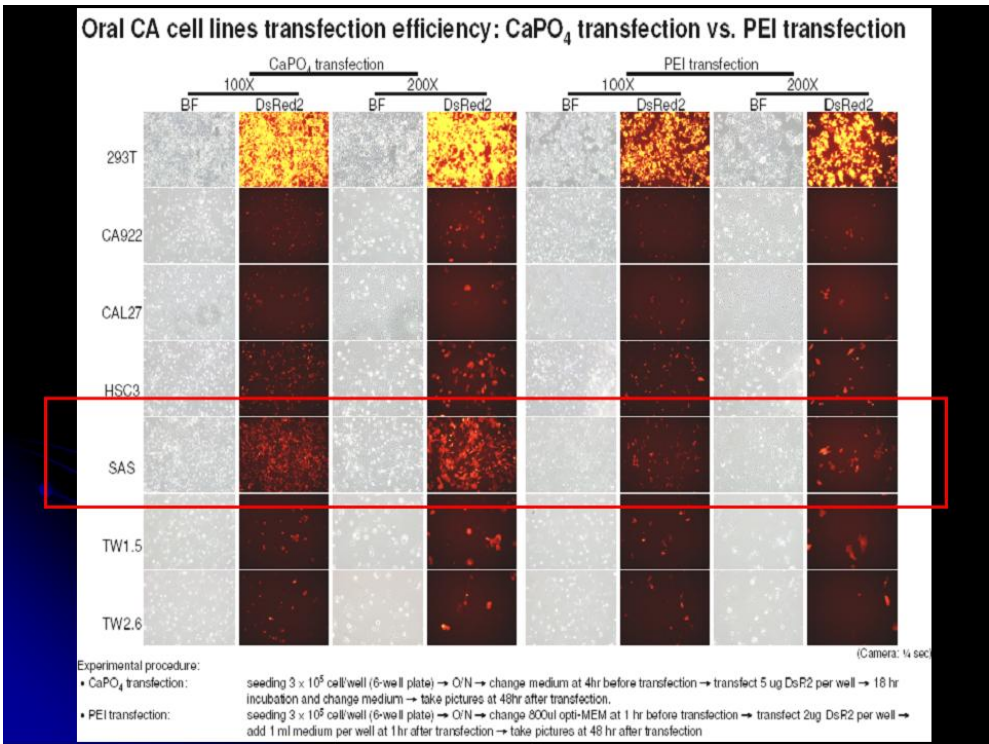


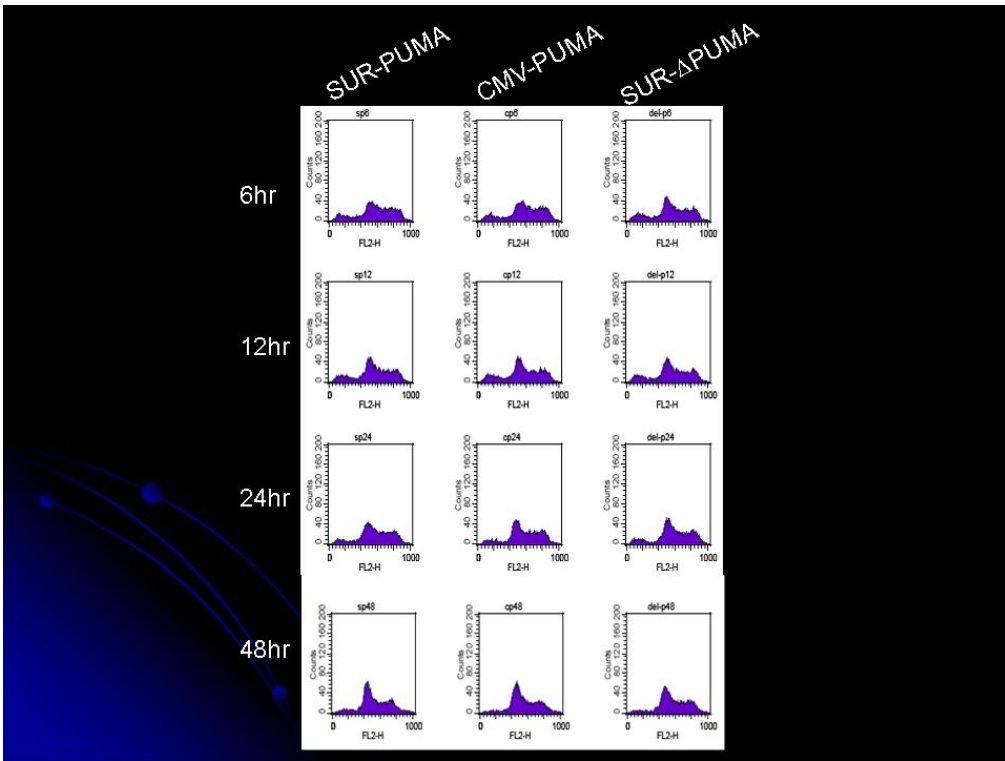
Rationale



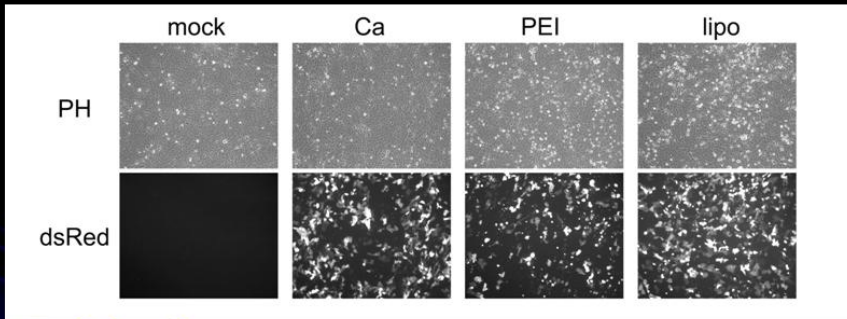
Constructs







Transfection efficiency



Time-course analysis of sur-PUMA in SAS

