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The Elk1 Gene Effect on Prostate Cancer Cell Line Wound Healing Ability

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Abstract
In this study prostate cancer cell line model was used selecting three type of them (Pc3, DU145 & LNCaP) , gene transfecting was done by using the liposome into the cell culture cells then detecting the healing ability by the Wound Healing or (scratching) test that shows clear effect of ELK1 gene in this cells comparing with the control cell transfected with the knock down gene, using for each type of cells 6 repeats ,for each type there was two groups 1st for control ELK1 and the 2nd was knock down or (sh) ELK1, all works was done in Johns Hopkins University / School of Medicine / Pathology Department (MD, USA)

Introduction
The scratch assay was performed to compare the influence control ELK1 plasmid against sh ELk1 (knock down),that was transfected to the three prostate cancer cell type that we used in our study DU145, Pc3 & LNCaP cells were seeded in a high density at 12-well plates, and grown till they reach more than 80% influence then transfected with the control ELK1 plasmid against sh ELk1 (knock down), Then the monolayer cells were physically wounded by scratching the surface with a pipette tip (200 µl) as uniformly and straight as possible. The images of cells invading the scratch were captured at indicated time points (24 hr.) using Inverted Microscope model CK40 Culture Microscope. The pictures were evaluated by measuring the difference in the area of the wounds with a ImageJ software and migration rate expressed as percentage of scratch closure was calculated as follows: % of scratch closure= a-b/a, where (a) is a distance between edges of the wound, and (b) is the distance which remained cell-free during cell migration to close the wound. The experiments were repeated in triplicate wells at least two times.(5)
In other word using 3 type of prostate cancer cell line PC3 , Du145 & LNCaP , then compareing between the control plasmid of ELK1 and the knock down sh ELK1 for each type we toke three picture for each well (12 well plate divide into 6 for control & 6 for sh) that mean 36 picture for each type of cell 3 repeats for each well then we transfer to excel taking the mean for each and by using program software called Image J for analyzing each one take the mean for each 6 of sh & the other 6 of control for comparing. (1,2)
cells are seeded into a multi-well assay plate and allowed to attach, spread, and form a confluent monolayer. A pin tool or needle is used to scratch and remove cells from a discrete area of the confluent monolayer to form a cell-free zone into which cells at the edges of the wound can migrate. (3,4)

Materials and methods
The prostate cancer cell line culture stocks from (ATCC Company / USA)
All transfection was done by using lipofectamin2000 of Invitrogen / USA
Growing medium for prostate cancer cell line was RPMI from Dako /USA
Elk-1 shRNA Plasmid (h), Elk-1 (h)-PR from SantaCruz Biotechnology / USA
Wound healing Assay Protocol
Prior to the assay:
1) Cells should be cultured to confluence or near (>90%) confluence in either 6 well dishes or 35 mM dishes.
2) Depending on the conditions, cell should be rinsed with PBS and starved in low serum media (1.5 ml; 0.5% - 0.1% serum in DMEM) overnight.
On the day of the assay:
3) Prepare 10ml of base media with any additives. Sterile filter the mixtures and place in sterile 15 ml falcon tubes. Store at 4oC. Warm up before using.
4) Draw a line with a marker on the bottom of the dish.
5) Using a sterile 200 µl pipet tip, scratch three separate wounds through the cells moving perpendicular to the line drawn in the step above. See the figure for arraignment of the scratches.
6) Rinse the cells (very gently as sheets of the cells may lift off if you are not careful) with PBS and replace with 1.5 ml of media containing any additives (agonist, inhibitors, activators, ect…).
7) Take a picture using phase contrast and 10X. Do this just above and just below each line. This will help orient your measurements. Make certain the line just appears in each picture. Name each picture 6hr2LA– This indicates the 6th hour, dish 2, Left scratch (vs right or center) above the line (vs B for below).
8) Take another picture at 6, 12 and 24 hours. After each measurement, replace the old media with the media/additives that you prepared in step 3.

All data in the table 1 & figure (1-3)and images(1-6)shows that the transfection work successfully and there is an effect of ELK1 on the wound healing or cell activity and motility through wound healing. It appears that the ELK1 plasmid increased the wound healing process in vitro. (2)

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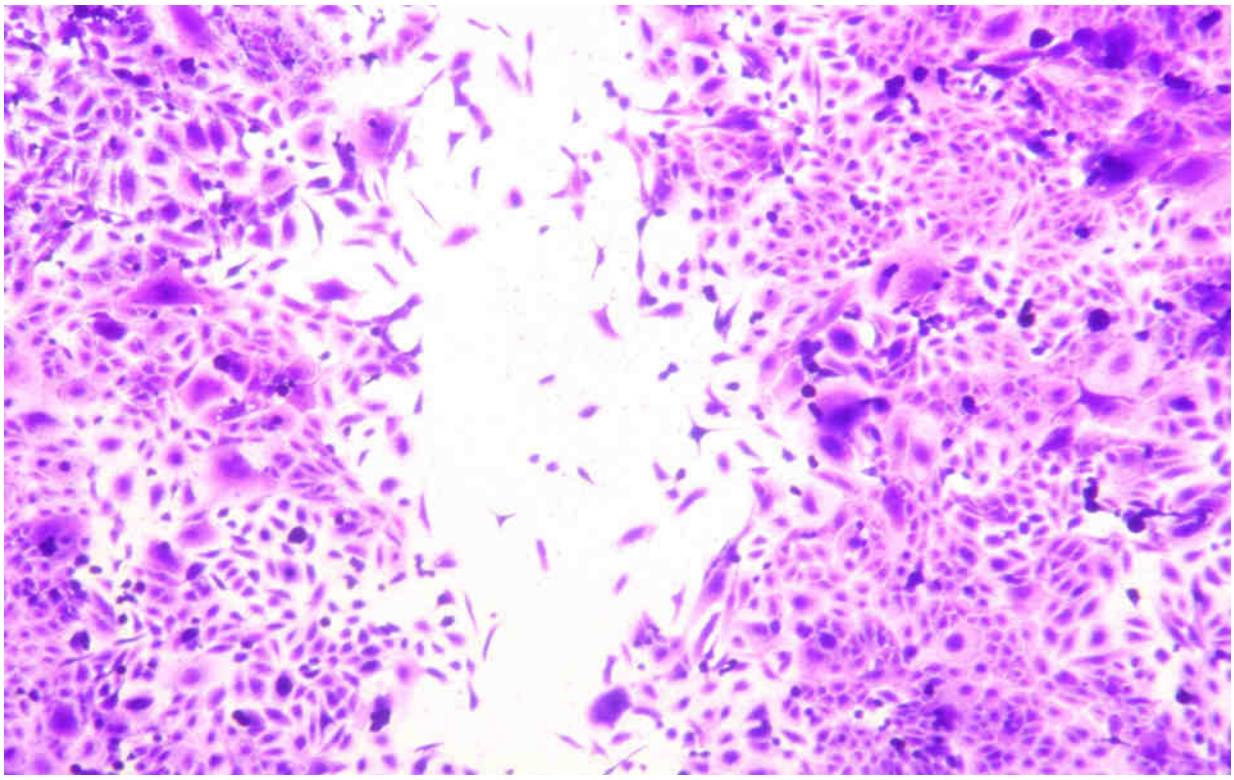

Image 1 PC3 control ELK1 Wound Healing Assay

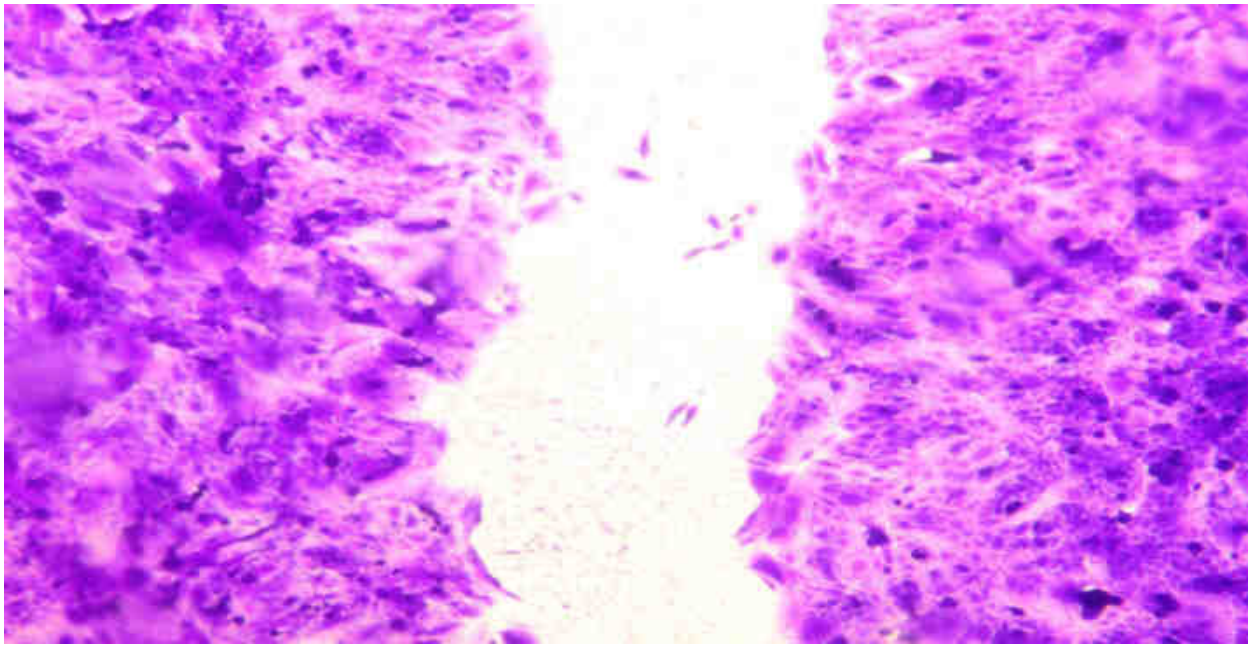

Image 2 PC3 sh ELK1 Wound healing assay.

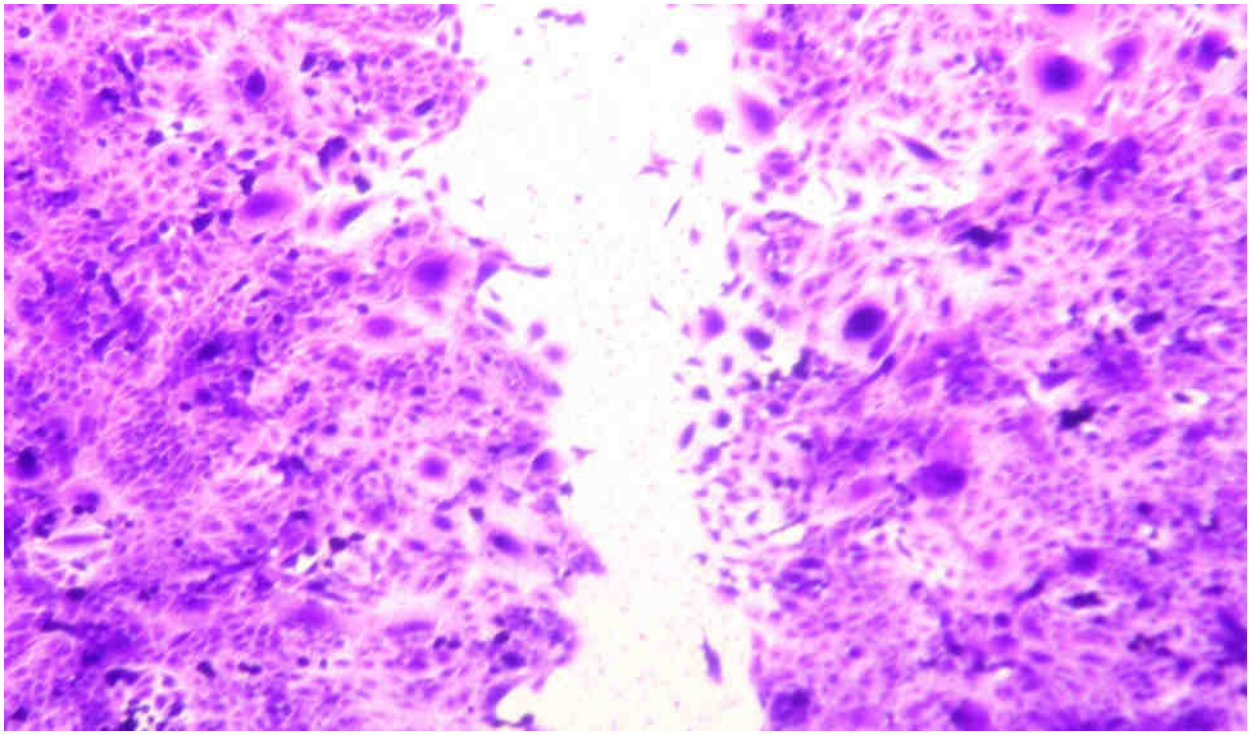

Image 3 DU145 control ELK1 Wound Healing Assay

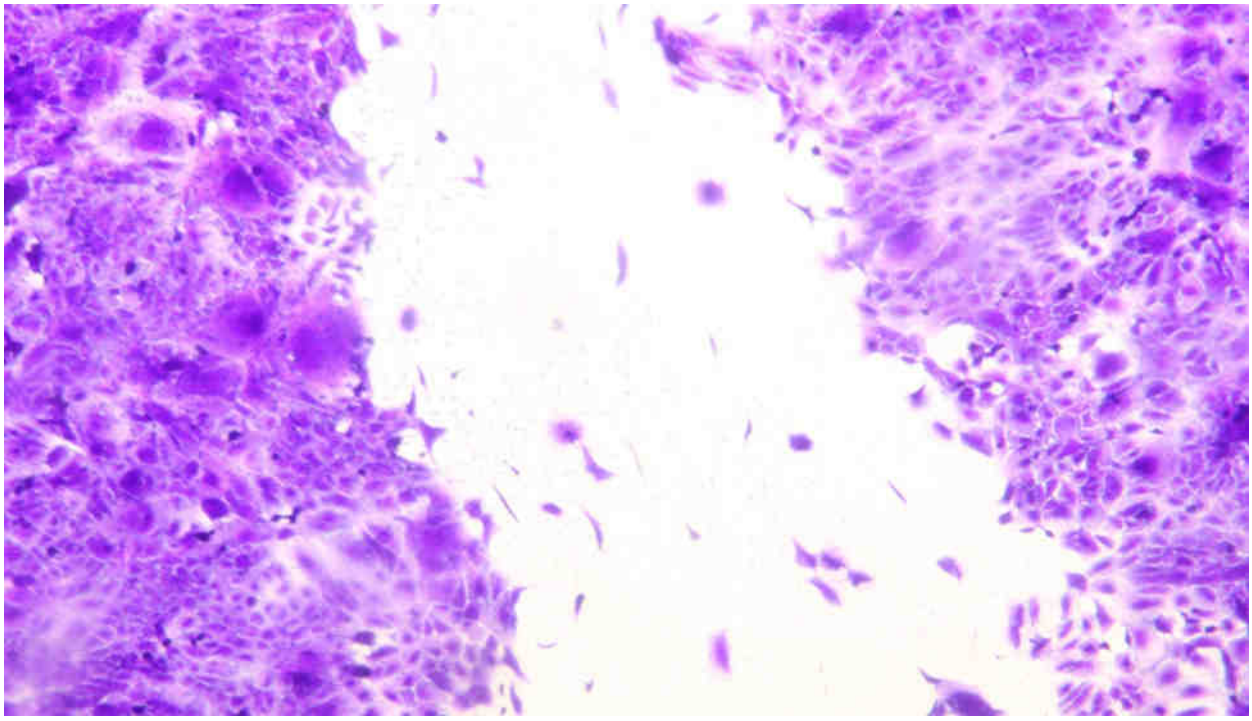

Image 4 DU145 sh ELK1 Wound healing Assay.

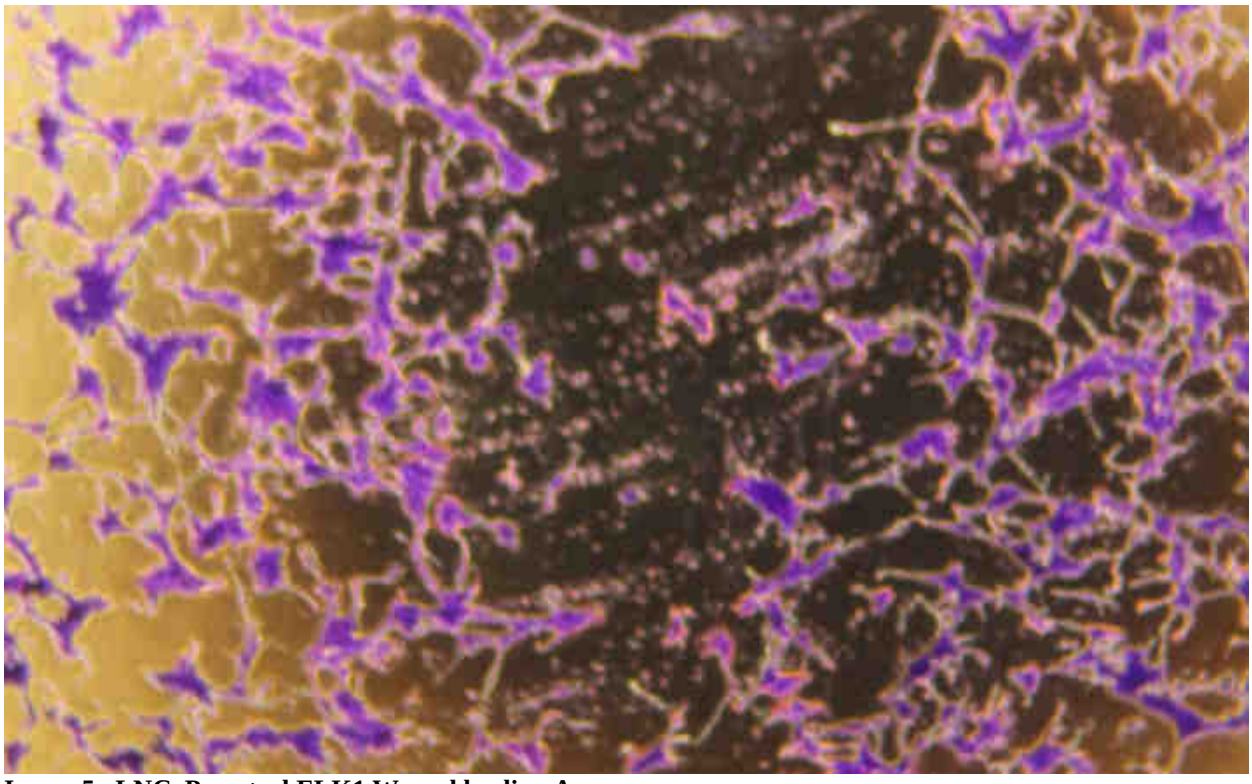

Image 5 LNCaP control ELK1 Wound healing Assay.

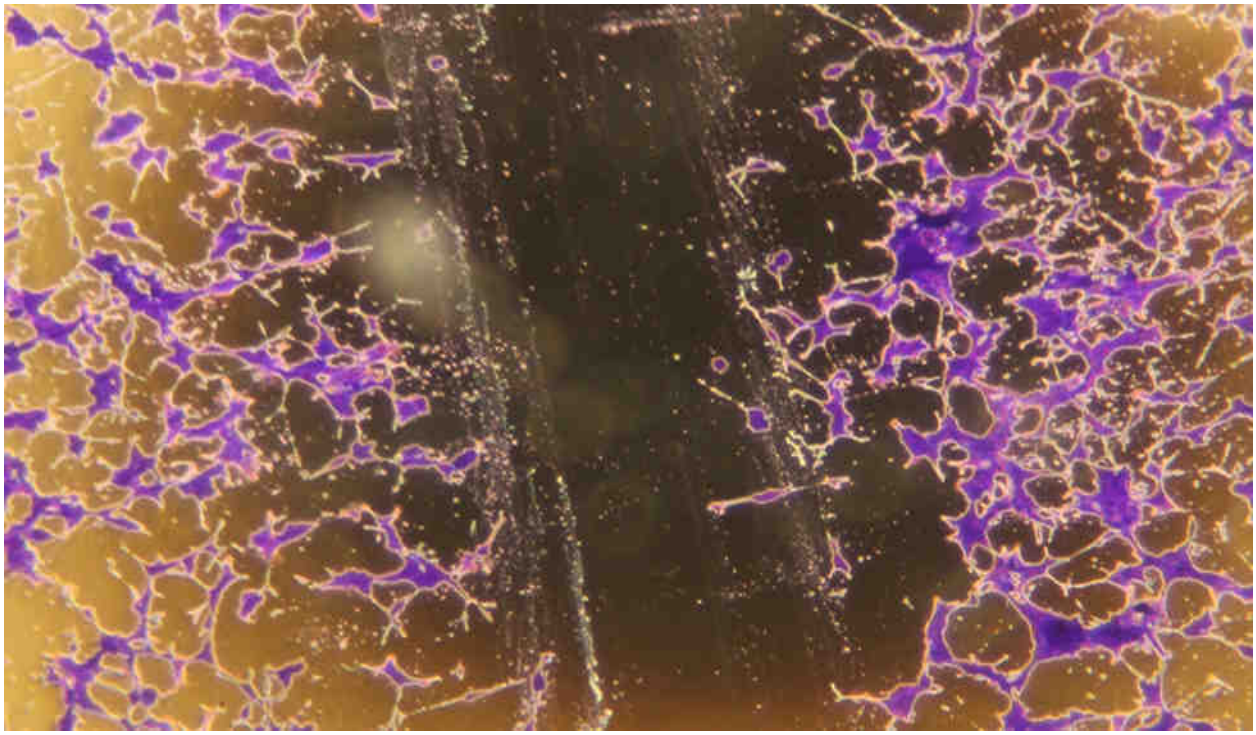

Image 6 LNCaP sh ELK1 Wound healing Assay.

	LNCp sh	LNCp cnt				PC3 sh	PC3 cnt					DU145 sh	DU145 cnt
	3469797	5300791				5037529	6010370					4833102	5600816
	3669401	5230216				4944600	6640238					4876391	5329059
	2775242	6487616				4470478	5558974					4767944	5419296
	1824584	6125196				4516682	5870506					4765964	5731847
	2566316	6604850				4583343	6647592					4813130	5746266
	2889487	5913863				4442849	6638753					4885306	5402351
	1122050	6221210				4259391	6884338					5000661	5833237
	1730245	5570636				4481029	6476777					4762429	5728015
	3146177	6725612				4759655	6830729					4399056	5764990
	2416456	4887504				4893455	5730711					4703838	5596357
	3547748	4667124				4711455	5806532					4845784	5781577
	2529084	5214381				4744192	5163852					4503297	5395777
mean	2640548.917	5745749.9				4653721.5	6188281					4763075.167	5610799
ratio	0.459565584	1				0.752021684	1					0.848912101	1
std	784706.9424	695678.41				233399.4268	566635.96					165697.9831	179698.3
stdr	0.136571719	0.121077				0.037716359	0.091566					0.029531976	0.0320272
p value	8.82401E-10					3.7916E-07						4.23548E-11	

Table 1 Wound Healing Assay result data for DU145, PC3 & LNCaP prostate cancer cell lines.

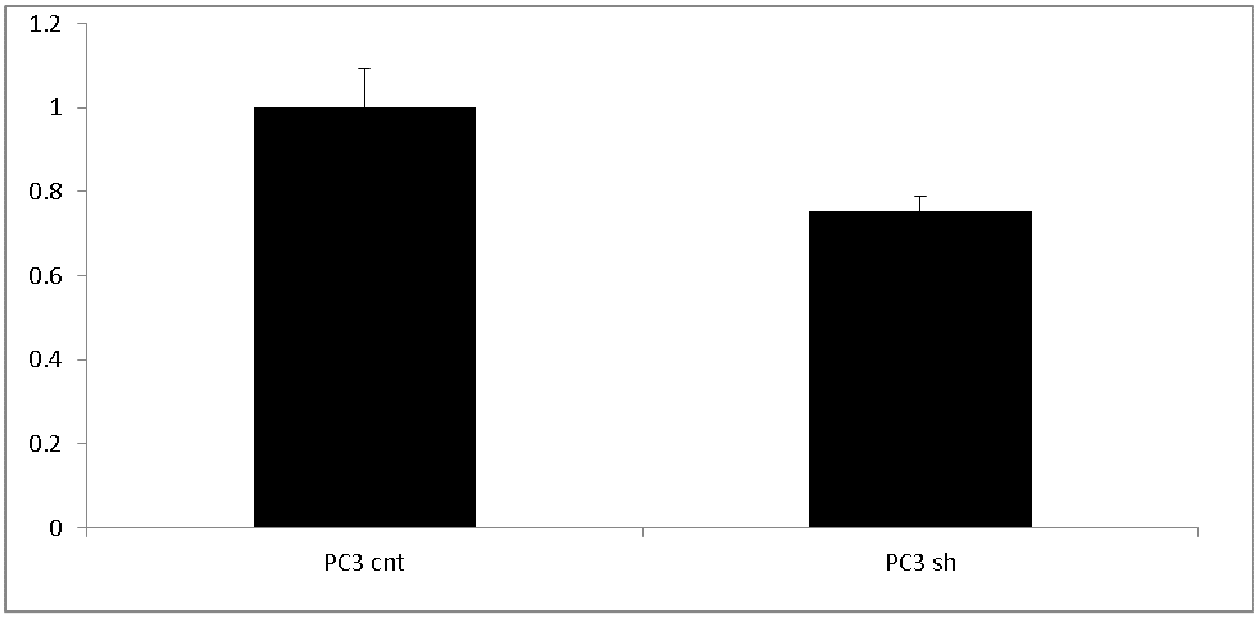

Figure 1 Comparison between the two plasmid of ELK1 (sh & control) on PC3 prostate cancer cells for wound healing assay

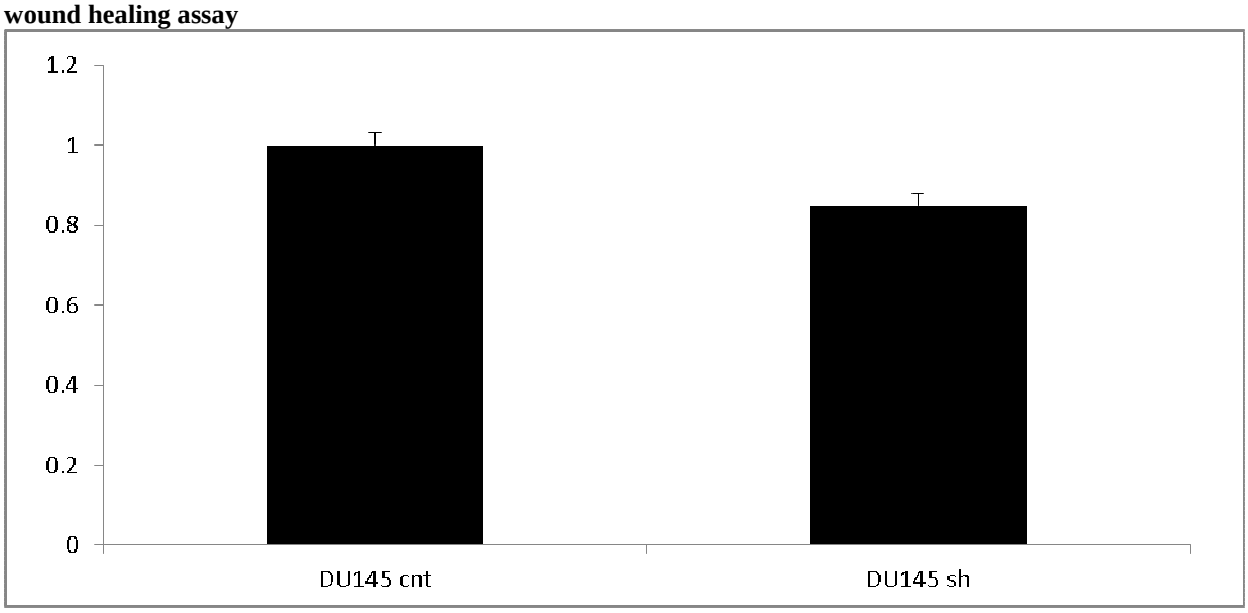

Figure 2 Comparison between the two plasmid of ELK1 (sh & control) on DU145 prostate cancer cells for wound healing assay

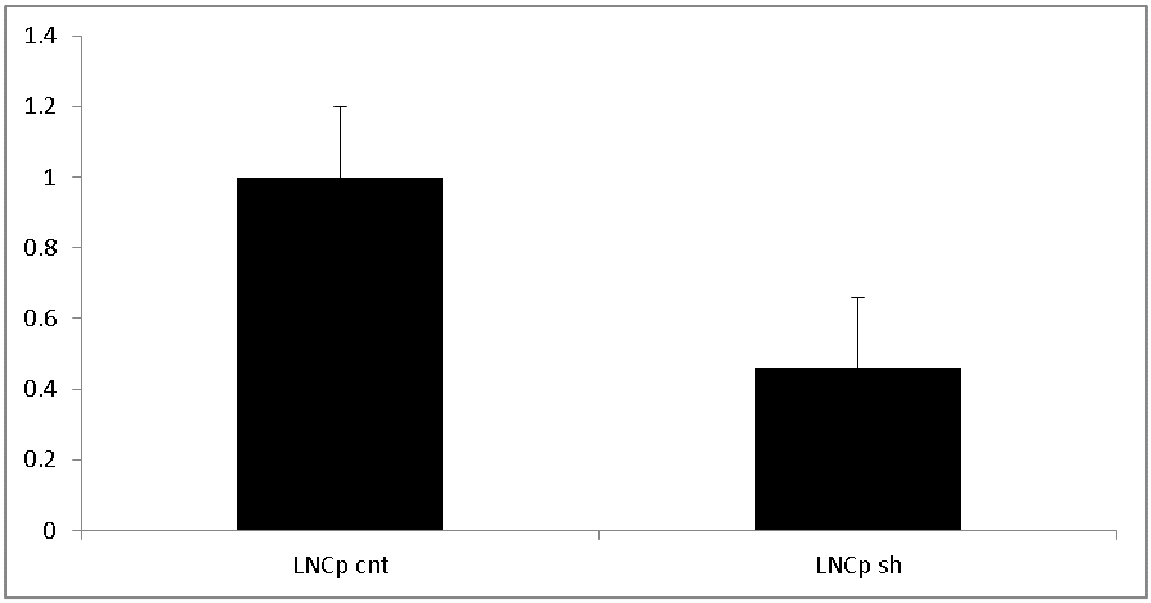

Figure 3 Comparison between the two plasmid of ELK1 (sh & control) on LNCaP prostate cancer cells for wound healing assay

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