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ELK1 Gene Transfection Effect in Prostate Cancer Cell Line Proliferation Activity

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Abstract
Three type of prostate cancer cell line was selected for this study (PC3, DU145 & LNCaP) as a model, transfected by liposome with the ELK1 gene (control & knock down) , then detecting the proliferation ability of the cultured cell lines by the Mtt or (proliferation) assay 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

MTT Proliferation Assay

DU145 , PC3 & LNCaP prostate cancer cells that previously transfected with the tow plasmids (control & sh) ELK1 were grow in 96-well plate each with containing normal media (RPMI and 10% FBS) at a density of $1*10^4$ cells /well for each cell type we divide the 96 –well plate into two parts one part for sh ELK1 and the other part is for control ELK1, for each type of cell we measure the proliferation for continuous five days and we measure the proliferation per day starting from Day 1 till Day 5 measuring was done by (Omega Fluostar of BMG Labtech) The absorbance was read at the wavelength of 570 nm and all steps are done as it mentioned in chapter three in 3.3.1.D [(1-4)] uses a chamber separated into two compartments by a porous filter membrane to allow the study of cells migration in response to gene transfection[(7)]. In this assay, the cells are seeded on one side of the membrane, while a solution to be tested for chemotactic activity is placed on the opposing side. After an incubation period, the membrane is fixed and stained. The number of cells which have migrated through the pores to the underside of the membrane in response to the chemotactic agent is counted microscopically [(7)]. Inhibitors of chemotaxis can be tested for activity by including them in one or both of the assay compartments. This assay has been broadly commercialized by several manufacturers such as BD Biosciences, Costar and R&D Systems as product lines of disposable transmembrane inserts for 24- or 96-well tissue culture plates. The membranes are available with different pore sizes to accommodate different cell types and with several choices of ECM coatings for use as a model of invasion [(6)] Ogasawra and co-workers [(8)] utilized Costar Transwell cell culture chambers in a 24-well format to screen 75 types of natural compounds from a variety of chemical classes for their ability to inhibit migration of the murine colon adenocarcinoma cell line 26-L5. The undersides of filters with 8 μm pores were coated with fibronectin and colon 26-L5 cells were pretreated with agents for 30 minutes prior to adding them to the upper compartment. Agents were added to the lower compartment at the same concentrations and after a 3 hour incubation to allow for migration, the cells were fixed and stained with 0.5% crystal violet for 30 minutes. Cells that migrated to the lower surfaces of the filters [(6)]

Results

The result in the tables and figures showed that the transfection of our tow plasmids (control & sh) ELK1 is successful and it have an effect on prostate cancer cell line proliferation in vitro , the proliferation rate is notably detected in case of using the control ELK1 plasmid is greater than it in the case of using the sh ELK1 plasmid with different rate for each cell type that we used in our experiment.[(4,5)]

Materials and methods

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
BD BioCoat™ Matrigel™ Invasion Chambers from Corning & Sigma Aldrich
All measurement was done by using the Omega Fluostar BMG Labtech machine
MTT assay (Proliferation Assay) protocol
1- Harvest suspension cells by centrifugation. Adherent cells should be released from their substrate by trypsinization or scraping.
2 - Resuspended cells at 1 x 106 per mL.
3 - Prepare serial dilutions of cells in culture medium from 1 x 106 to 1 x 103 cells per mL.

4 - Plate out, in triplicate, 100 µL of the dilutions into wells of a microtiter plate.
5 - Include three control wells of medium alone to provide the blanks for absorbance readings.
6 - Incubate the cells under conditions appropriate for the cell line for 6 to 48 hours (to recover from handling). The time required will vary but 12 hours to overnight is sufficient for most cell types.
7 - Add 10 µL of MTT Reagent to each well, including controls.
8 - Return plate to cell culture incubator for 2 to 4 hours.
9- Periodically view the cells under an inverted microscope for presence of intracellular punctate purple precipitate.
10 - When the purple precipitate is clearly visible under the microscope add 100 µL of Detergent Reagent to all wells, including controls. Swirl gently; do not shake.
11 - Leave plate with cover in the dark for 2 to 4 hours or overnight at room temperature.
12- Remove plate cover and measure the absorbance in each well, including the blanks, at 570 nm in a microtiter plate reader. Absorbance's can be read with any filter in the wavelength range of 550 - 600 nm. The reference wavelength should be higher than 650 nm. The blanks should give values close to zero (+/- 0.1).]
13- If the readings are low return the plate to the dark for longer incubation.
14 - Determine the average values from triplicate readings and subtract the average value for the blank. Plot absorbance against number of cells/mL. The number of cells to use in your assay should lie within the linear portion of the plot and yield an absorbance of 0.75 - 1.25.

References.

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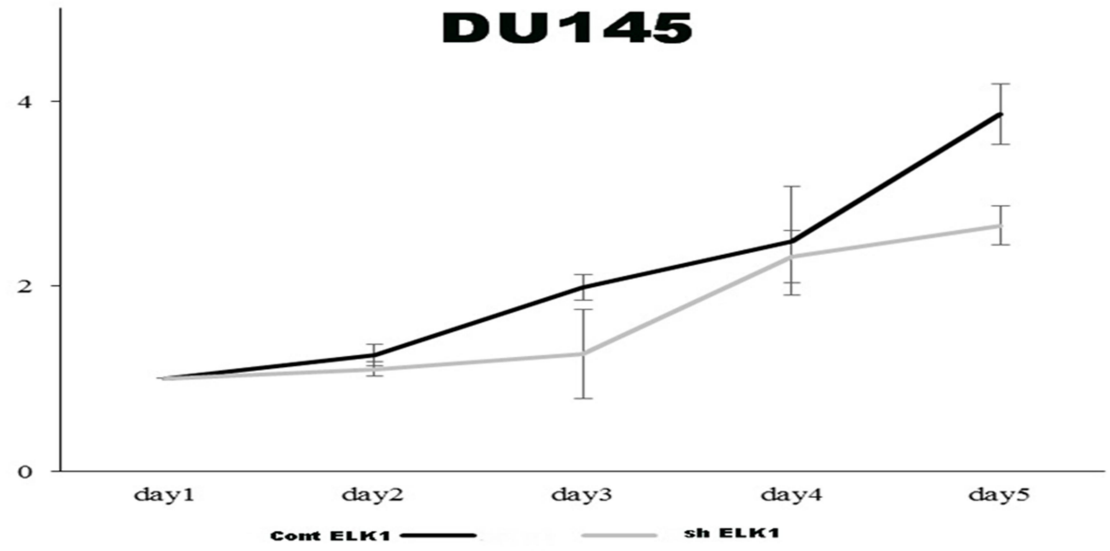

Figure 1 MTT Proliferation assay for the DU145 cells.

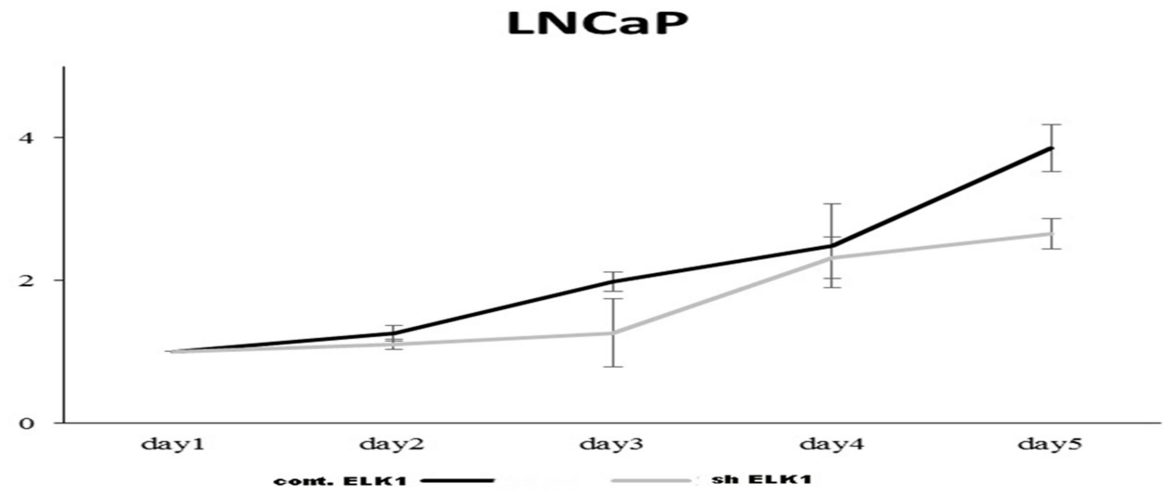

Figure 2 MTT Proliferation assay for the LNCaP cells.

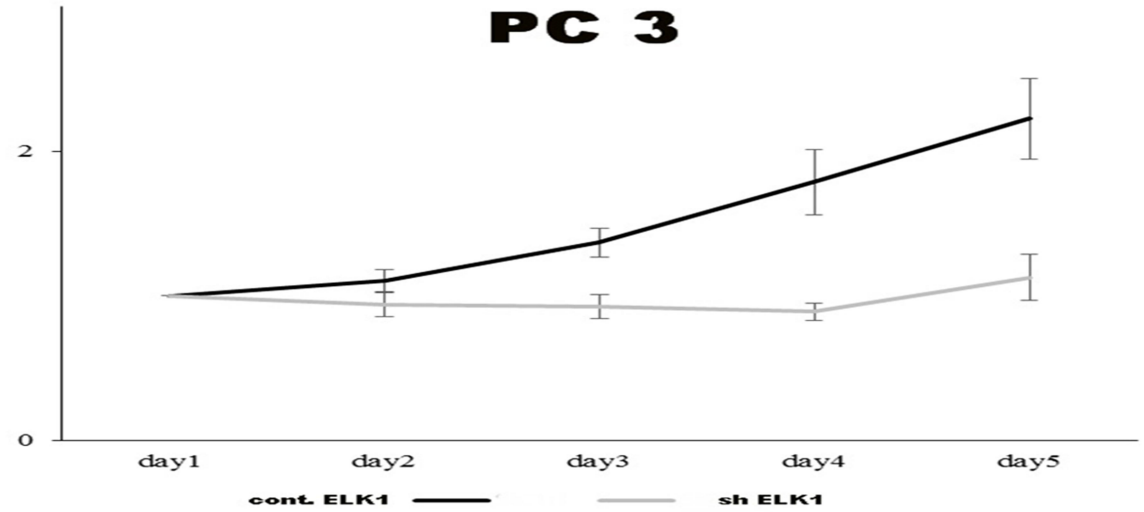

Figure 3 MTT Proliferation assay for the PC3 cells.

	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
1	0.2275	0.2565	0.23363	0.26138	0.438	0.55525	0.75343	0.52114	0.86675	0.68075		
2	0.21988	0.23825	0.28675	0.25013	0.42063	0.27875	0.64257	0.59343	0.88563	0.61438		
3	0.21525	0.23738	0.287	0.29113	0.46888	0.31925	0.58729	0.65543	0.88925	0.6545		
4	0.225	0.24975	0.29125	0.2645	0.40288	0.27888	0.42929	0.51557	0.94525	0.59213		
5	0.23225	0.249	0.30663	0.27188	0.47438	0.195	0.507	0.53629	0.7805	0.63625		
6	0.2315	0.23225	0.2905	0.26725	0.47388	0.22988	0.434	0.556	0.832	0.697		
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	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
1	1	1	1.026923	1.019006	1.925275	2.164717	3.311774	2.031746	3.80989	2.653996	0	0
2	1	1	1.30415	1.049843	1.913019	1.16999	2.92244	2.490781	4.027857	2.578699	0	0
3	1	1	1.333333	1.226435	2.178281	1.344918	2.728389	2.761152	4.131243	2.757241	0	0
4	1	1	1.294444	1.059059	1.790556	1.116617	1.907937	2.06435	4.201111	2.370871	0	0
5	1	1	1.320237	1.091867	2.042519	0.783133	2.182992	2.153758	3.360603	2.555221	0	0
6	1	1	1.25486	1.1507	2.046976	0.989774	1.87473	2.393972	3.593952	3.001076	0	0
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	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
ave	0.22523	0.24385	0.28263	0.26771	0.44644	0.3095	0.55893	0.56298	0.86656	0.64583	#DIV/0!	#DIV/0!
stdev	0.00666	0.00926	0.0251	0.01361	0.03057	0.12792	0.12708	0.05335	0.05598	0.03969	#DIV/0!	#DIV/0!
p	*	0.00307	*	0.23778	*	0.04645	*	0.94475	*	2.5E-05	*	#DIV/0!

	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
ave	1	1	1.255658	1.099485	1.982771	1.261525	2.488044	2.31596	3.854109	2.652851	0	0
stdev	0	0	0.115222	0.076646	0.134933	0.480582	0.588342	0.284689	0.328812	0.212933	0	0
p	*	#DIV/0!	*	**0.02265**	*	**0.013001**	*	**0.538928**	*	**4.77E-05**	*	**#DIV/0!**
%				0.875625		0.636243		0.930836		0.688317		#DIV/0!

RAW	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
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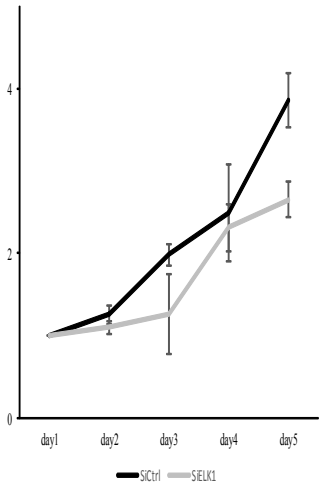

ave	day1	day2	day3	day4	day5
SiCtrl	1	1.255658	1.982771	2.488044	3.854109
SiELK1	1	1.099485	1.261525	2.31596	2.652851

stdev	day1	day2	day3	day4	day5	day6
SiCtrl	0	0.115222	0.134933	0.588342	0.328812	0
SiELK1	0	0.076646	0.480582	0.284689	0.212933	0

SiCtrl	SiELK1
3.80989	2.653996
4.027857	2.578699
4.131243	2.757241
4.201111	2.370871
3.360603	2.555221
3.593952	3.001076

Table 1 MTT Proliferation assay data result for the DU145 cell.

	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
1	0.1815	0.226	0.199	0.18113	0.26625	0.178	0.38088	0.18638	0.4095	0.3035		
2	0.1795	0.03	0.20513	0.18225	0.25175	0.18213	0.34263	0.18275	0.394	0.1855		
3	0.17988	0.198	0.2	0.18338	0.23888	0.19025	0.33338	0.18438	0.37938	0.18425		
4	0.20313	0.199	0.19488	0.19538	0.24063	0.19013	0.30075	0.19238	0.35963	0.2035		
5	0.18025	0.20075	0.21025	0.19688	0.24788	0.183	0.28588	0.17113	0.44538	0.24363		
6	0.1925	0.21138	0.223	0.211	0.27838	0.21225	0.34538	0.18338	0.49125	0.23813		
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	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
1	1	1	1.096419	0.801438	1.466942	0.787611	2.098485	0.824668	2.256198	1.34292	0	0
2	1		1.142758		1.402507		1.908774		2.194986		0	0
3	1	1	1.111883	0.926136	1.328006	0.960859	1.85337	0.931187	2.109104	0.930556	0	0
4	1	1	0.959385	0.981784	1.184615	0.955402	1.480615	0.966709	1.770462	1.022613	0	0
5	1	1	1.166436	0.980697	1.375173	0.911582	1.585992	0.852428	2.470874	1.213574	0	0
6	1	1	1.158442	0.998226	1.446104	1.00414	1.794156	0.867534	2.551948	1.126552	0	0
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	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
ave	0.18613	0.17752	0.20538	0.19167	0.25396	0.18929	0.33148	0.1834	0.41319	0.22642	#DIV/0!	#DIV/0!
stdev	0.00968	0.07305	0.01014	0.01169	0.01546	0.01222	0.03401	0.00695	0.04801	0.0455	#DIV/0!	#DIV/0!
p	*	0.78596	*	0.05572	*	1.5E-05	*	8.6E-05	*	4.2E-05	*	#DIV/0!

	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
ave	1	1	1.105887	0.937656	1.367225	0.923918	1.786899	0.888505	2.225595	1.127243	0	0
stdev	0	0	0.07665	0.080865	0.102331	0.082951	0.223868	0.058639	0.279019	0.160938	0	0
p	*	#DIV/0!	*	**0.007224**	*	**2.36E-05**	*	**9.68E-05**	*	**3.39E-05**	*	**#DIV/0!**
%				0.847877		0.675762		0.497233		0.506491		#DIV/0!

RAW	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
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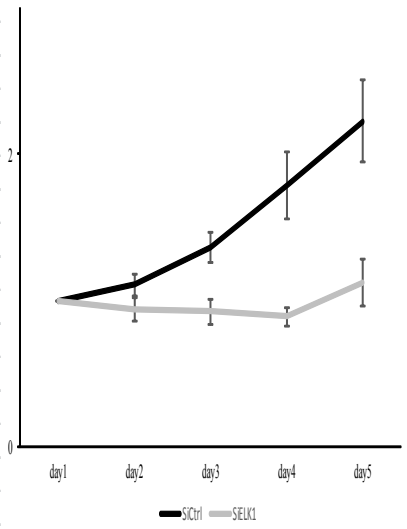

ave	day1	day2	day3	day4	day5
SiCtrl	1	1.105887	1.367225	1.786899	2.225595
SiELK1	1	0.937656	0.923918	0.888505	1.127243

stdev	day1	day2	day3	day4	day5	day6
SiCtrl	0	0.07665	0.102331	0.223868	0.279019	0
SiELK1	0	0.080865	0.082951	0.058639	0.160938	0

Table 2 MTT Proliferation assay data result for the PC3 cells.

	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
1	0.2275	0.2565	0.23363	0.26138	0.438	0.55525	0.75343	0.52114	0.86675	0.68075		
2	0.21988	0.23825	0.28675	0.25013	0.42063	0.27875	0.64257	0.59343	0.88563	0.61438		
3	0.21525	0.23738	0.287	0.29113	0.46888	0.31925	0.58729	0.65543	0.88925	0.6545		
4	0.225	0.24975	0.29125	0.2645	0.40288	0.27888	0.42929	0.51557	0.94525	0.59213		
5	0.23225	0.249	0.30663	0.27188	0.47438	0.195	0.507	0.53629	0.7805	0.63625		
6	0.2315	0.23225	0.2905	0.26725	0.47388	0.22988	0.434	0.556	0.832	0.697		
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	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
1	1	1	1.026923	1.019006	1.925275	2.164717	3.311774	2.031746	3.80989	2.653996	0	0
2	1	1	1.30415	1.049843	1.913019	1.16999	2.92244	2.490781	4.027857	2.578699	0	0
3	1	1	1.333333	1.226435	2.178281	1.344918	2.728389	2.761152	4.131243	2.757241	0	0
4	1	1	1.294444	1.059059	1.790556	1.116617	1.907937	2.06435	4.201111	2.370871	0	0
5	1	1	1.320237	1.091867	2.042519	0.783133	2.182992	2.153758	3.360603	2.555221	0	0
6	1	1	1.25486	1.1507	2.046976	0.989774	1.87473	2.393972	3.593952	3.001076	0	0
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	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
ave	0.22523	0.24385	0.28263	0.26771	0.44644	0.3095	0.55893	0.56298	0.86656	0.64583	#DIV/0!	#DIV/0!
stdev	0.00666	0.00926	0.0251	0.01361	0.03057	0.12792	0.12708	0.05335	0.05598	0.03969	#DIV/0!	#DIV/0!
p	*	0.00307	*	0.23778	*	0.04645	*	0.94475	*	2.5E-05	*	#DIV/0!

	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
ave	1	1	1.255658	1.099485	1.982771	1.261525	2.488044	2.31596	3.854109	2.652851	0	0
stdev	0	0	0.115222	0.076646	0.134933	0.480582	0.588342	0.284689	0.328812	0.212933	0	0
p	*	#DIV/0!	*	**0.02265**	*	**0.013001**	*	**0.538928**	*	**4.77E-05**	*	**#DIV/0!**
%				0.875625		0.636243		0.930836		0.688317		#DIV/0!

RAW	day1		day2		day3		day4		day5		day6	
	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1	SiCtrl	SiELK1
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2												
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5												
6												
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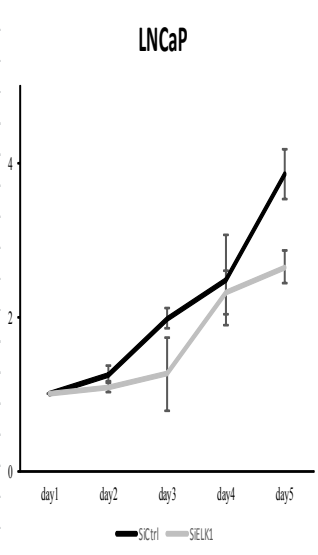
ave	day1	day2	day3	day4	day5
SiCtrl	1	1.255658	1.982771	2.488044	3.854109
SiELK1	1	1.099485	1.261525	2.31596	2.652851

stdev	day1	day2	day3	day4	day5	day6
SiCtrl	0	0.115222	0.134933	0.588342	0.328812	0
SiELK1	0	0.076646	0.480582	0.284689	0.212933	0



SiCtrl	SiELK1
3.80989	2.653996
4.027857	2.578699
4.131243	2.757241
4.201111	2.370871
3.360603	2.555221
3.593952	3.001076

Table 3 MTT Proliferation assay data result for the LNCaP cells.

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