A STUDY ON ANTI-CANCEROUS CAPABILITIES OF PTP7 ON DIFFERENT CELL LINES BY MTT ASSAY

Science

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ABSTRACT

In the present cancer advancing days, scientific investigation was catch up in studying the protein peptides involving in signalling pathways in regulating cancer cell lines development and proliferation. In our current we focused on an essential peptide protein tyrosine phosphatase, which plays an important role in signalling path way. This work evaluated the efficiency of PTP7 against 3 cancer cell lines renal, breast and prostate cancer lines by MTT assay. PTP 7 on Renal cancer cell line (HTB9) shown 73.660%, breast cancer cells (MFC-7) shown 79.313% and Prostate cancer cells (PC-3) shown 87.559 % of viability by MTT assay. The advancing of protein tyrosine protease is concluded to have good viability over can cell lines, as it can further investigated in controlling of cancer.

KEYWORDS

Protein-tyrosine Phosphatase (ptp7), Mtt Assay, Cancer Lines Htb9, Mfc-7 And Pc-3.

INTRODUCTION

Protein-tyrosine phosphatase (PTPs), regulates important signalling pathways which are involved in control of adhesion, cell proliferation and migration. Perturbation of PTK activity by mutations or over express only results in malignant transformation, and PTK inhibitors are now established (Roman *et al.*, 2012).

Anticancer drugs. In 1988, the first PTP was purified, approx. 10 years after the discovery of tyrosine kinases³. It constitute diverse family of tightly regulated and highly specific enzymes with important regulatory. As emphasized below, it is also clear that PTPs have both inhibitory as well as stimulatory effects on signalling processes associated to cancer, and deregulation of PTP function is associated with tumorigenesis in different types of human cancer (Ruckert MT *et al.*, 2019). PTPs are broadly divided into receptor-like and non-receptor forms. The receptor-like PTPs show a single trans membrane domain and variable number of extracellular domains. The intracellular parts of the receptor-like PTPs contain two tandem PTP domains (D and D2) with most, if not all, of the catalytic activity residing in D1. In many cases, the extracellular domains include immunoglobulin like domains and fibro nectin type III domains, similar to extracellular domains of cellular adhesion molecules. Non-receptor PTPs have striking structure & diversity and usually contain sequences that target them to a specific subcellular location or enable binding to specific proteins (Johnson KG *et al.*, 2003). The catalytic PTP domain spans approximately 280 amino acids and contains highly conserved active sites with a cysteine residue that is required for catalytic activity. Dephosphorylation of substrates occurs via a two-step mechanism consisting of the formation of covalent PTP-phosphate intermediate that is subsequently hydrolyzed. Dephosphorylation by PTPs occurs with a very high degree of specificity, as demonstrated by the fold difference the KM for various phosphor peptides and by selective substrate-binding of substrate trapping PTP variants. Some studies have also shown that PTP preferentially dephosphorylate certain subsets of phosphorylated tyrosine's on proteins that have multiple phosphorylation sites. Substrate specificity has at least two determinants; first, significant side-chain interactions occur between the substrates and areas that flank the PTP active site; and subcellular compartmentalization restricts the substrate access. Since their discovery PTPs have been considered potential tumour suppressors because of the antagonistic effects on oncogenic signalling of PTK. Early experimentation support for this was provided by over expression of PTPs in cells, which induced reversion of tyrosine-kinase-dependent transformation. The design of PTP inhibitors has been challenging because they often share

a common reaction mechanism utilizing a conserved Cysteine as well as other features of the active site. The EYA families of PTPs act by a distinct mechanism using an Aspartate as a nucleophile. Hence they serve as an attractive new target specific to breast cancer and possibly to other cancer too. A number of potential PTP inhibitors (inhibiting greater than 80% and some of them greater has been identified which will serve as a great therapeutic agent. (Hendriks WJAJ., 2008)

METHODOLOGY:

MTT ASSAY:

- The anticancer activities of the peptides on different cell lines were determined by the MTT assay (Mosmann, 1983).
- In 96-well plates the cells (1×10^5 /well) were plated as 0.2 ml of medium/well and incubated at 5% CO₂ in an incubator for 72 h.
- Then, various concentrations of the samples were added in 0.1% DMSO and incubated for 48 h at 5% CO₂.
- The sample solution was removed and washed with phosphate-buffered saline (pH 7.4), then 20 μ l/well (5 mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) in phosphate buffered saline solution were added.
- Then 1 ml of DMSO was added after 4 h of incubation.
- Viable cells were determined by absorbance at 540 nm. Measurements were executed and the concentrations required for a 50% inhibition of viability (IC₅₀) were determined graphically.
- The effects of the samples on the proliferation of cells were expressed as the % cell viability by using the following formula:

$$\% \text{ cell viability} = \frac{A_{540} \text{ of treated cells}}{A_{540} \text{ of control cells}} \times 100$$

RESULTS:

To evaluate the cytotoxic activity of PTP7 on three cell lines renal cancer cell lines (HTB9), breast cancer cell lines (MFC-7) and prostate cancer cell lines (PC-3) MTT assay was performed. The dosage of PTP7 ranges from 50-1000 μ g/ml with an increment of 50 μ g/ml and incubated for 72 hours while readings were taken for every 24 hours. Cell viability of the cells was recorded as the anticancer peptide showed activity in a concentration dependent manner. At all concentrations of the anticancer peptide showed activity against the cell lines by inhibiting their growth. IC₅₀ was calculated for the activity of PTP7 on all cell lines and recorded to be 912.7 μ g/ml, 942.4 μ g/ml and 604.1 μ g/ml for HTB9, MFC-7 and PC-3 cell lines respectively.

A protein peptide protein tyrosine protease efficiency of its viability control was examined against three can cell lines include Renal cancer cell line (HTB9), breast cancer cells (MFC-7) and Prostate cancer cells (PC-3).

Table represents Cell Viability test by MTT assay of PTP7 on Renal cancer cell line (HTB9)

		24 Hours	48 Hours	72 Hours	Percentage Cell Viability		
Control	PCM	0.941	0.855	0.391	24 Hours	48 Hours	72 Hours
PTP7K1	S1D1	0.955	0.633	0.308	96.430	84.210	73.660
	S1D2	0.835	0.770	0.297			
	S1D3	0.933	0.757	0.259			

	Average	0.9074063	0.720	0.288			
		0.907	0.720	0.288			
PTP7K2	S1D1	0.906	0.573	0.212	97.310	86.300	69.440
	S1D2	0.812	0.659	0.216			
	S1D3	0.931	0.631	0.174			
	Average	0.8830854	0.621	0.200			
		0.883	0.621	0.200			
PTP7K3	S1D1	0.892	0.511	0.151	92.540	87.590	74.620
	S1D2	0.727	0.560	0.156			
	S1D3	0.824	0.561	0.141			
	Average	0.8172072	0.544	0.150			
		0.815	0.544	0.149			
PTP7K4	S3D1	0.785	0.504	0.163	96.28	85.99	75.83
	S3D2	0.908	0.524	0.149			
	S3D3	0.925	0.452	0.167			
	Average	0.873	0.493	0.161			
		0.873	0.493	0.160			
PTP7K5	S1D1	0.913	0.405	0.122	95.830	87.510	73.990
	S1D2	0.715	0.449	0.121			
	S1D3	0.877	0.436	0.112			
	Average	0.83629	0.431	0.118			
		0.835	0.430	0.118			
PTP7K6	S3D1	1.482	0.812	0.734	82.83	77.19	61.53
	S3D2	1.726	0.790	0.630			
	S3D3	1.696	0.672	0.705			
	Average	1.634	0.758	0.690			
		1.634	0.758	0.689			
PTP7K7	S3D1	0.752	0.322	0.079	89.53	73.61	61.75
	S3D2	0.858	0.320	0.070			
	S3D3	0.842	0.253	0.078			
	Average	0.818	0.298	0.075			
		0.818	0.298	0.076			
PTP7K8	S3D1	1.114	0.637	0.409	86.42	75.33	52.91
	S3D2	1.371	0.643	0.358			
	S3D3	1.358	0.555	0.397			
	Average	1.281	0.612	0.388			
		1.281	0.612	0.388			
PTP7K9	S3D1	0.731	0.641	0.591	83.77	70.69	65.83
	S3D2	0.799	0.630	0.507			
	S3D3	0.787	0.559	0.569			
	Average	0.772	0.610	0.556			
		0.772	0.610	0.556			
PTP7K10	S1D1	0.710	0.470	0.459	78.320	62.890	53.710
	S1D2	0.589	0.558	0.442			
	S1D3	0.704	0.523	0.362			
	Average	0.6688528	0.517	0.421			
		0.668	0.517	0.421			
PTP7K11	S3D1	0.583	0.406	0.315	85.82	61.35	50.16
	S3D2	0.655	0.418	0.268			
	S3D3	0.643	0.356	0.309			
	Average	0.627	0.393	0.296			
		0.627	0.393	0.297			
PTP7K12	S3D1	0.650	0.548	0.411	81.673	65.31	49.72
	S3D2	0.725	0.570	0.364			
	S3D3	0.715	0.494	0.394			
	Average	0.697	0.537	0.390			
		0.697	0.537	0.390			
PTP7K13	S3D1	0.412	0.239	0.146	80.42	57.13	43.88
	S3D2	0.483	0.239	0.129			
	S3D3	0.509	0.217	0.141			
	Average	0.469	0.232	0.138			
		0.468	0.232	0.139			
PTP7K14	S1D1	0.391	0.145	0.063	79.554	65.840	43.861
	S1D2	0.338	0.157	0.066			
	S1D3	0.382	0.157	0.055			
	Average	0.3723937	0.153	0.061			
		0.371	0.153	0.061			
PTP7K15	S3D1	0.497	0.481	0.378	69.83	52.27	38.692
	S3D2	0.555	0.495	0.339			
	S3D3	0.567	0.422	0.370			
	Average	0.540	0.466	0.363			
		0.539	0.466	0.362			

PTP7K16	S3D1	0.265	0.079	0.021	74.62	52.97	32.126
	S3D2	0.300	0.080	0.020			
	S3D3	0.309	0.070	0.021			
	Average	0.292	0.077	0.020			
		0.291	0.076	0.021			
PTP7K17	S1D1	0.208	0.030	0.007	68.550	46.810	32.730
	S1D2	0.184	0.038	0.007			
	S1D3	0.208	0.038	0.007			
	Average	0.1996622	0.036	0.007			
		0.200	0.036	0.007			
PTP7K18	S1D1	0.125	0.018	0.003	60.180	53.970	42.810
	S1D2	0.113	0.020	0.003			
	S1D3	0.124	0.021	0.003			
	Average	0.1202018	0.019	0.003			
		0.121	0.019	0.003			
PTP7K19	S3D1	0.107	0.014	0.002	59.38	43.72	31.62
	S3D2	0.132	0.014	0.002			
	S3D3	0.131	0.012	0.002			
	Average	0.124	0.013	0.002			
		0.123	0.013	0.002			
PTP7K20	S2D1	0.474	0.324	0.181	52.27	38.365	17.41
	S2D2	0.404	0.324	0.181			
	S2D3	0.495	0.256	0.157			
	Average	0.457	0.301	0.173			
		0.457	0.301	0.173			

Graph 1 Graphical representation of PTP7 against HTB9 cell line

DISCUSSION:

Table represents viability of renal cancer cells decreases with increasing concentrations of PTP7. The percentage of cell viability decreases with increasing concentrations of PTP7. The percentage of cell viability decreases from 73.66% to 17.41% by exposing cell lines to PTP7 at different intervals of time. This preliminary test indicates the significant role of PTP7 on controlling the growth of cancer cells.

Table represents Cell Viability test by MTT assay of PTP7 on breast cancer cells (MFC-7):

		24 Hours	48 Hours	72 Hours	Percentage Cell Viability		
Control	PCM	0.941	0.855	0.391	24 Hours	48 Hours	72 Hours
PTP7B1	S1D1	0.984	0.736	0.313	95.720	91.684	79.313
	S1D2	0.801	0.807	0.322			
	S1D3	0.908	0.808	0.292			
	Average	0.9007252	0.784	0.310			
		0.898	0.784	0.309			
PTP7B2	S1D1	0.862	0.595	0.262	91.180	86.347	79.198
	S1D2	0.754	0.724	0.252			
	S1D3	0.843	0.712	0.221			
	Average	0.819228	0.677	0.245			
		0.819	0.677	0.245			
PTP7B3	S1D1	0.815	0.519	0.200	94.617	87.215	76.381
	S1D2	0.713	0.632	0.193			
	S1D3	0.797	0.621	0.168			
	Average	0.7752065	0.590	0.187			
		0.775	0.590	0.187			
PTP7B4	S1D1	1.931	0.754	0.887	89.642	81.734	76.831
	S1D2	1.512	0.835	0.879			
	S1D3	1.854	0.812	0.818			
	Average	1.7686367	0.803	0.861			
		1.767	0.801	0.861			
PTP7B5	S3D1	0.673	0.474	0.160	91.752	89.46	78.68
	S3D2	0.778	0.493	0.146			
	S3D3	0.793	0.426	0.164			

	Average	0.748	0.464	0.158			
		0.748	0.464	0.157			
PTP7B6	S3D1	1.514	0.843	0.837	83.39	79.42	70.83
	S3D2	1.728	0.836	0.742			
	S3D3	1.695	0.660	0.819			
	Average	1.645	0.780	0.794			
		1.645	0.780	0.798			
PTP7B7	S3D1	0.537	0.425	0.133	87.94	83.71	78.36
	S3D2	0.625	0.413	0.114			
	S3D3	0.614	0.352	0.128			
	Average	0.592	0.397	0.125			
		0.592	0.397	0.125			
PTP7B8	S3D1	1.169	0.672	0.609	81.57	75.84	68.37
	S3D2	1.279	0.660	0.522			
	S3D3	1.259	0.586	0.586			
	Average	1.235	0.640	0.572			
		1.236	0.640	0.572			
PTP7B9	S3D1	0.679	0.705	0.560	84.68	78.42	62.97
	S3D2	0.834	0.712	0.491			
	S3D3	0.827	0.614	0.543			
	Average	0.780	0.677	0.531			
		0.780	0.677	0.531			
PTP7B10	S1D1	0.668	0.487	0.490	73.612	65.239	57.328
	S1D2	0.553	0.579	0.472			
	S1D3	0.661	0.542	0.387			
	Average	0.6286465	0.536	0.449			
		0.628	0.536	0.449			
PTP7B11	S3D1	0.529	0.460	0.338	83.761	63.995	57.316
	S3D2	0.591	0.479	0.300			
	S3D3	0.583	0.415	0.325			
	Average	0.568	0.451	0.321			
		0.568	0.451	0.321			
PTP7B12	S1D1	0.461	0.270	0.198	77.317	62.956	59.215
	S1D2	0.399	0.293	0.205			
	S1D3	0.450	0.293	0.171			
	Average	0.4390212	0.284	0.190			
		0.437	0.285	0.191			
PTP7B13	S3D1	0.441	0.355	0.197	89.52	74.83	54.819
	S3D2	0.495	0.366	0.168			
	S3D3	0.486	0.312	0.193			
	Average	0.474	0.345	0.185			
		0.474	0.344	0.186			
PTP7B14	S3D1	0.311	0.191	0.119	76.943	68.527	56.874
	S3D2	0.365	0.191	0.105			
	S3D3	0.385	0.173	0.115			
	Average	0.355	0.185	0.112			
		0.354	0.185	0.113			
PTP7B15	S3D1	0.573	0.640	0.575	81.692	69.568	58.21
	S3D2	0.649	0.647	0.526			
	S3D3	0.668	0.568	0.565			
	Average	0.631	0.621	0.545			
		0.630	0.619	0.553			
PTP7B16	S3D1	0.224	0.114	0.058	79.455	57.853	46.827
	S3D2	0.254	0.115	0.054			
	S3D3	0.262	0.101	0.057			
	Average	0.247	0.110	0.055			
		0.247	0.110	0.056			
PTP7B17	S1D1	0.180	0.063	0.030	69.872	59.883	50.725
	S1D2	0.162	0.068	0.031			
	S1D3	0.177	0.070	0.027			
	Average	0.1725265	0.066	0.029			
		0.173	0.067	0.029			
PTP7B18	S3D1	0.452	0.523	0.302	72.934	64.729	49.351
	S3D2	0.504	0.538	0.271			
	S3D3	0.515	0.459	0.295			
	Average	0.491	0.507	0.290			
		0.491	0.507	0.290			
PTP7B19	S2D1	0.300	0.279	0.116	64.147	49.628	36.773
	S2D2	0.256	0.279	0.116			
	S2D3	0.313	0.221	0.101			
	Average	0.290	0.259	0.111			
		0.290	0.259	0.111			

PTP7B20	S1D1	0.162	0.118	0.045	53.792	53.723	39.659
	S1D2	0.143	0.149	0.047			
	S1D3	0.162	0.146	0.043			
	Average	0.1558214	0.139	0.044			

Graph 2: Graphical representation of PTP7 against PC 3 cell line

DISCUSSION:

Table represents viability of breast cancer cells decreases with increasing concentrations of PTP7. The percentage of cell viability decreases with increasing concentrations of PTP7. The percentage of cell viability decreases from 79.313% to 39.659% by exposing cell lines to PTP7 at different intervals of time. This preliminary test indicates the significant role of PTP7 on controlling the growth of cancer cell

Table represents Cell Viability test by MTT assay on Prostate cancer cells (PC-3)

PTP7P1	S1D1	0.954	0.672	0.353	97.325	92.651	87.559
	S1D2	0.842	0.840	0.363			
	S1D3	0.952	0.864	0.312			
	Average	0.9158283	0.792	0.342			
PTP7P2	S2D1	0.919	0.823	0.353	94.288	89.583	86.3
	S2D2	0.784	0.823	0.353			
	S2D3	0.959	0.652	0.307			
	Average	0.887	0.766	0.337			
PTP7P3	S3D1	0.631	0.571	0.201	72.934	64.729	49.351
	S3D2	0.705	0.588	0.180			
	S3D3	0.721	0.501	0.197			
	Average	0.686	0.553	0.193			
PTP7P4	S1D1	1.436	0.564	0.591	69.872	59.883	50.725
	S1D2	1.295	0.606	0.614			
	S1D3	1.418	0.630	0.546			
	Average	1.3785746	0.588	0.569			
PTP7P5	S3D1	0.455	0.340	0.099	79.455	57.853	46.827
	S3D2	0.516	0.344	0.091			
	S3D3	0.531	0.302	0.098			
	Average	0.502	0.330	0.094			
PTP7P6	S3D1	1.463	0.704	0.688	81.692	69.568	58.21
	S3D2	1.657	0.712	0.630			
	S3D3	1.705	0.625	0.675			
	Average	1.612	0.683	0.653			
LFB6K7	S3D1	0.308	0.241	0.060	76.943	68.527	56.874
	S3D2	0.361	0.241	0.053			
	S3D3	0.380	0.218	0.058			
	Average	0.350	0.233	0.057			
PTP7P8	S3D1	1.218	0.544	0.401	89.52	74.83	54.819
	S3D2	1.369	0.560	0.341			
	S3D3	1.342	0.477	0.392			
	Average	1.310	0.527	0.377			
PTP7P9	S1D1	1.063	0.315	0.233	77.317	62.956	59.215
	S1D2	0.920	0.342	0.242			
	S1D3	1.039	0.342	0.201			
	Average	1.0126142	0.332	0.224			
PTP7P10	S3D1	0.950	0.355	0.242	83.761	63.995	57.316
	S3D2	1.061	0.369	0.215			
	S3D3	1.046	0.320	0.232			

	Average	1.020	0.348	0.230			
		1.019	0.348	0.230			
PTP7P11	S3D1	0.783	0.257	0.154	84.68	78.42	62.97
	S3D2	0.963	0.260	0.135			
	S3D3	0.954	0.224	0.150			
	Average	0.900	0.247	0.147			
		0.900	0.247	0.147			
PTP7P12	S3D1	0.660	0.655	0.570	81.57	75.84	68.37
	S3D2	0.722	0.643	0.489			
	S3D3	0.711	0.571	0.549			
	Average	0.697	0.623	0.536			
		0.697	0.623	0.536			
PTP7P13	S3D1	0.625	0.230	0.129	87.94	83.71	78.36
	S3D2	0.727	0.224	0.111			
	S3D3	0.715	0.191	0.124			
	Average	0.689	0.215	0.121			
		0.689	0.215	0.121			
PTP7P14	S3D1	0.506	0.562	0.426	83.39	79.42	70.83
	S3D2	0.578	0.558	0.378			
	S3D3	0.567	0.441	0.416			
	Average	0.550	0.520	0.404			
		0.550	0.520	0.406			
PTP7P15	S3D1	0.638	0.815	0.747	91.752	89.46	78.68
	S3D2	0.738	0.847	0.682			
	S3D3	0.752	0.732	0.768			
	Average	0.709	0.798	0.737			
		0.709	0.798	0.734			
PTP7P16	S3D1	0.365	0.336	0.210	79.455	57.853	46.827
	S3D2	0.413	0.339	0.192			
	S3D3	0.425	0.298	0.206			
	Average	0.402	0.325	0.199			
		0.401	0.324	0.202			
PTP7P17	S1D1	0.420	0.279	0.162	95.720	91.684	79.313
	S1D2	0.342	0.306	0.167			
	S1D3	0.388	0.307	0.151			
	Average	0.3842792	0.297	0.160			
		0.383	0.297	0.160			
PTP7P18	S1D1	0.368	0.226	0.135	91.180	86.347	79.198
	S1D2	0.322	0.275	0.130			
	S1D3	0.359	0.270	0.114			
	Average	0.3495098	0.257	0.127			
		0.350	0.257	0.127			
PTP7P19	S1D1	0.871	0.602	0.813	94.617	87.215	76.381
	S1D2	0.762	0.732	0.782			
	S1D3	0.851	0.720	0.683			
	Average	0.8278988	0.685	0.759			
		0.828	0.685	0.759			
PTP7P20	S1D1	0.810	0.525	0.601	89.642	81.734	76.831
	S1D2	0.635	0.582	0.595			
	S1D3	0.778	0.566	0.554			
	Average	0.7422192	0.560	0.583			
		0.741	0.558	0.583			

Graph 3: Graphical representation of PTP7 against PC 3 cell line

IC50 of PTP7 against PC-3 cell line

DISCUSSION:

Table represents viability of prostate cancer cells decreases with increasing concentrations of PTP7. The percentage of cell viability decreases with increasing concentrations of PTP7. The percentage of cell viability decreases from 87.559% to 76.831% by exposing cell lines to PTP7 at different intervals of time. This preliminary test indicates the significant role of PTP7 on controlling the growth of cancer cells.

CONCLUSION:

The results clearly executed the effective peptide PTP7 against the viability control upon cancer lines. Viability percentage values expressed indicated its signalling control efficiency over its growth. PTP7 on Renal cancer cell line (HTB9) shown 73.660%, breast cancer cells (MFC-7) shown 79.313% and Prostate cancer cells (PC-3) shown 87.559% of viability by MTT assay.

Therapeutic approaches in cancer treatment has majorly confined. This specific activity of PTP7 can unlock the door way to a whole new generation of cancer therapeutics if explored in an organized and with

systematic methods. Global industrial support as well government funding is abundant in this particular domain once a systematic method is been developed for peptide based cancer therapy.

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