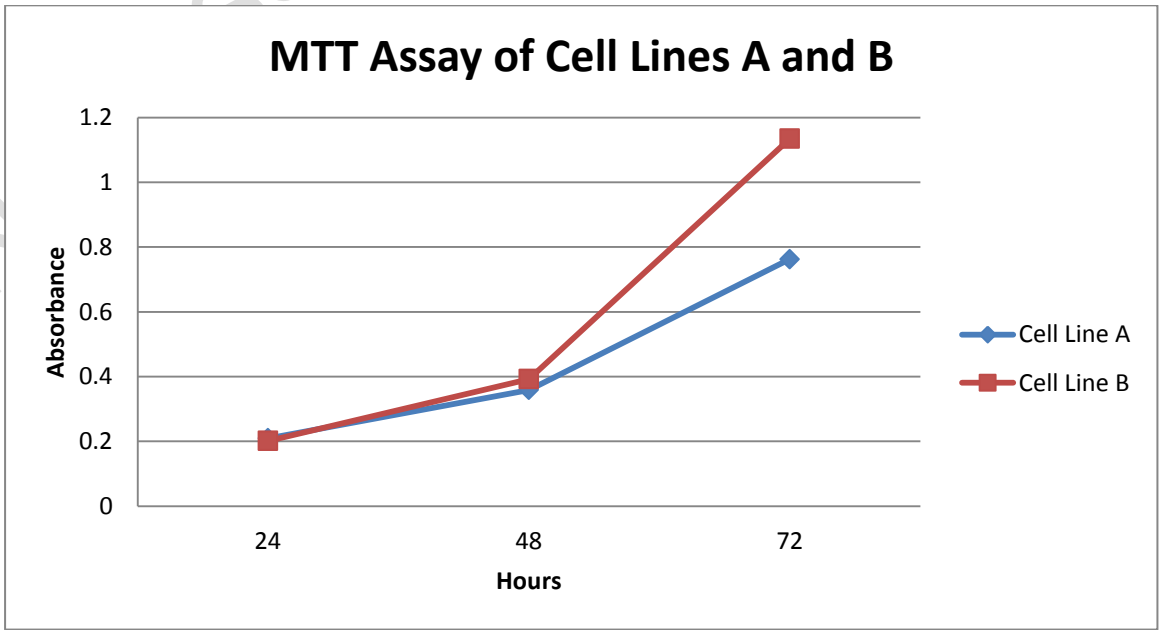
Part A: Results of the Study

Is there a difference of curve profile between the MTT assay and the cell number? What do the different tests determine, and what does it mean regarding your results?

The following Figures 1 and 2 identify the comparison of curve profile of the two different cell lines, A and B. It is observed that the growth rate of cell line B is much higher than the growth rate of cell line A. The curve profile is similar for both the cell count test and the MTT assay.

In the MTT assay, the tetrazolium dye reaction is dependent on the level of NAD(P)H-dependent oxidoreductase enzyme present in the cytosol of the cell. This enzyme reduces the dye to form formazan, whose concentration is assessed using calorimetry. An important point to be noted in this process is that the reduction of the MTT is dependent on the level of metabolic activity. Viable but metabolically at rest cells reduce little MTT, while rapidly dividing cells like cancer cell are found to show high rates of reduction. Therefore, the MTT assay can be considered as a method used to identify the viability of cells as well as the rate of division (i.e. metabolic activity).

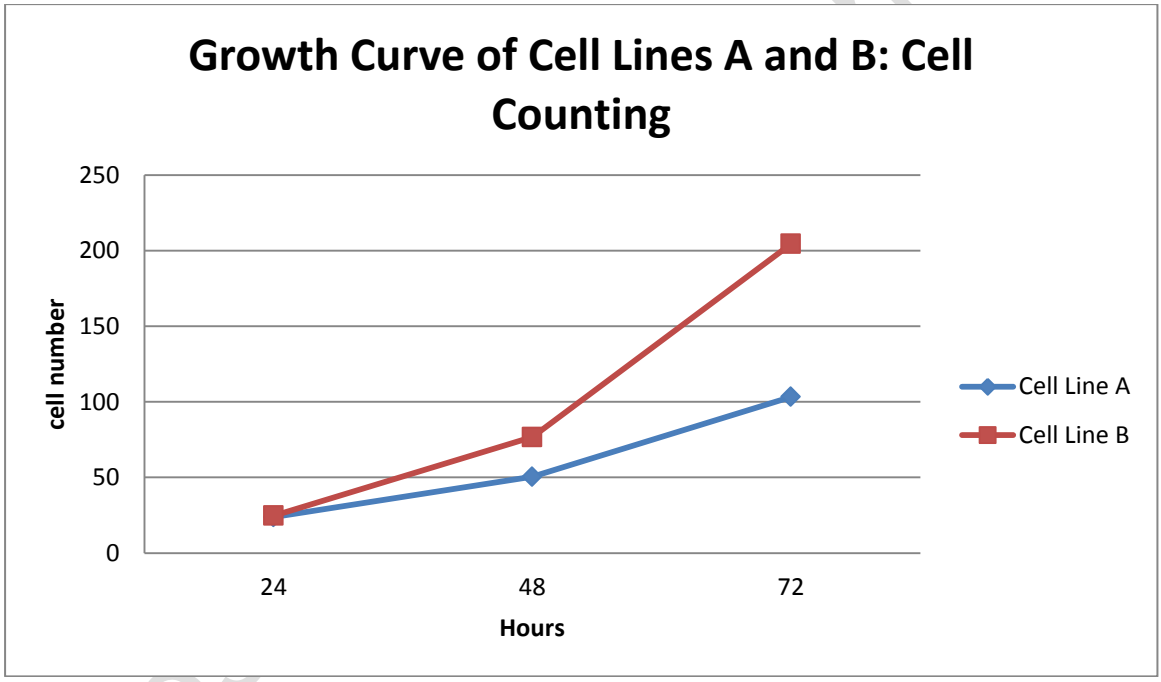
Figure 1: MTT Assay



In the cell counting method, the dye uptake by viable cells is identified. Therefore, an increase in the number of whole cells with time will show a greater number of cells on the haemocytometer. Therefore, the cell counting method provides information only on the viability of the cells.

It is concluded that, since both the tests identify the total number of viable cells, the curve profile between the tests should be similar.

Figure 2: Cell Count of Cell Line A and B



Do you see any changes in cell growth between the two different cell lines? What are your conclusions?

The changes in cell growth between the two cell lines can be compared by conducting an independent sample t-test and a one sample t-test. The purpose of the one sample t-test is to identify the mean cell number and absorbance at different time intervals. Table 1 identifies the one sample t-test for cell number, while Table 2 presents the one sample t-test for MTT assay. It is observed from Table 1 that cell line A shows a lower absorbance at 24 hours (0.209), 48 hours

(0.358) and 72 hours (0.726) when compared to cell line B, which shows higher absorbance at 24 hours (0.205), 48 hours (0.388) and 72 hours (1.079).

Table 1: One Sample T-Test for MTT Assay Results

MTT Results for Cell Line A: T-Test

	N	Mean	Std. Deviation	T	df	Sig. (2-tailed)	Mean Difference
24 hour cell count	6	.2096	.00479	107.182	5	.000	.20958
48 hour cell count	6	.3587	.00386	227.856	5	.000	.35867
72 hour cell count	6	.7626	.03593	51.991	5	.000	.76258

MTT Results for Cell Line B: T-Test

	N	Mean	Std. Deviation	T	df	Sig. (2-tailed)	Mean Difference
24 hour cell count	6	.2053	.01107	45.411	5	.000	.20525
48 hour cell count	6	.3881	.01827	52.027	5	.000	.38808
72 hour cell count	6	1.0798	.13528	19.553	5	.000	1.07983

Table 2: One Sample T-Test for Cell Count Results

Cell Count Results for Cell Line A: T Test

	N	Mean	Std. Deviation	t	df	Sig. (2-tailed)	Mean Difference
24 hour	4	23.7500	3.30404	14.376	3	.001	23.75000
48 hour	4	50.5000	6.40312	15.774	3	.001	50.50000
72 hour	4	103.2500	8.46069	24.407	3	.000	103.25000

Cell Count Results for Cell Line B: T Test

	N	Mean	Std. Deviation	t	df	Sig. (2-tailed)	Mean Difference
24 hour	4	24.7500	2.75379	17.975	3	.000	24.75000
48 hour	4	76.7500	6.99405	21.947	3	.000	76.75000
72 hour	4	204.5000	16.60321	24.634	3	.000	204.50000

It is observed from Table 2 that cell line A shows a lower cell number at 24 hours (23.7), 48 hours (50.5) and 72 hours (103.25), when compared to cell line B which shows greater number at 24 hours (24.7), 48 hours (76.75) and 72 hours (204.5).

Table 3: Independent Sample T-Test: Comparing Cell Count at 24 and 72 hours

		Levene's Test for Equality of Variances		t-test for Equality of Means			
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference
Cell Count for A	Equal variances assumed	5.400	.059	-17.505	6	.000	-79.50000
	Equal variances not assumed			-17.505	3.894	.000	-79.50000
Cell Count for B	Equal variances assumed	3.417	.114	-21.361	6	.000	-179.75
	Equal variances not assumed			-21.361	3.165	.000	-179.75

Table 4: Independent Sample T-Test: Comparing MTT Assay at 24 and 72 hours

		Levene's Test for Equality of Variances		t-test for Equality of Means			
		F	Sig.	t	df	Sig.	Mean Difference
MTT Assay for A	Equal variances assumed	19.522	.001	-37.371	10	.000	-.553
	Equal variances not assumed			-37.371	5.178	.000	-.553
MTT Assay for B	Equal variances assumed	.242	.633	-94.819	10	.000	-.933
	Equal variances not assumed			-94.819	8.014	.000	-.933

From the independent sample t-test, it is observed that the mean difference between the cell absorbance at time 24 hours and 48 hours is 0.553 for cell line A and 0.993 for cell line B. Similarly, the mean difference for cell counting at time 24 hours and 48 hours is 79.75 for cell line A and 179.75 for cell line B. From the above analysis of T-test results, one can conclude that cell line B has a greater growth rate when compared to cell line A. For instance, consider the cell number or absorbance at time 24 hours. There is limited difference between the two lines. However, over the next 48 hours there is a significant increase in number of cells for cell line B when compared to cell line A.

Calculation of Percentage Viability

Cell Counting Assay

Incubation time{hr}	Number of viable cells {live cells}	Number of nonviable cells{dead cells}	Total Cell	% viability
24hr	5	9	14	35.71%
48hr	12	6	18	66.67%
72hr	18	11	29	62.07%

MTT Assay

Incubation time{hr}	Number of viable cells {live cells}	Number of nonviable cells{dead cells}	Total Cell	% viability
24hr	12	8	20	60.00%
48hr	19	13	32	59.38%
72hr	28	10	38	73.68%

From the above tables it is observed that the percentage of viable cells is found to increase with time.

Having a quick search of the literature on the characteristics of cancer cells, what kind of suggestions could you make to start to explain what is happening in the cells and how it affects their growth?

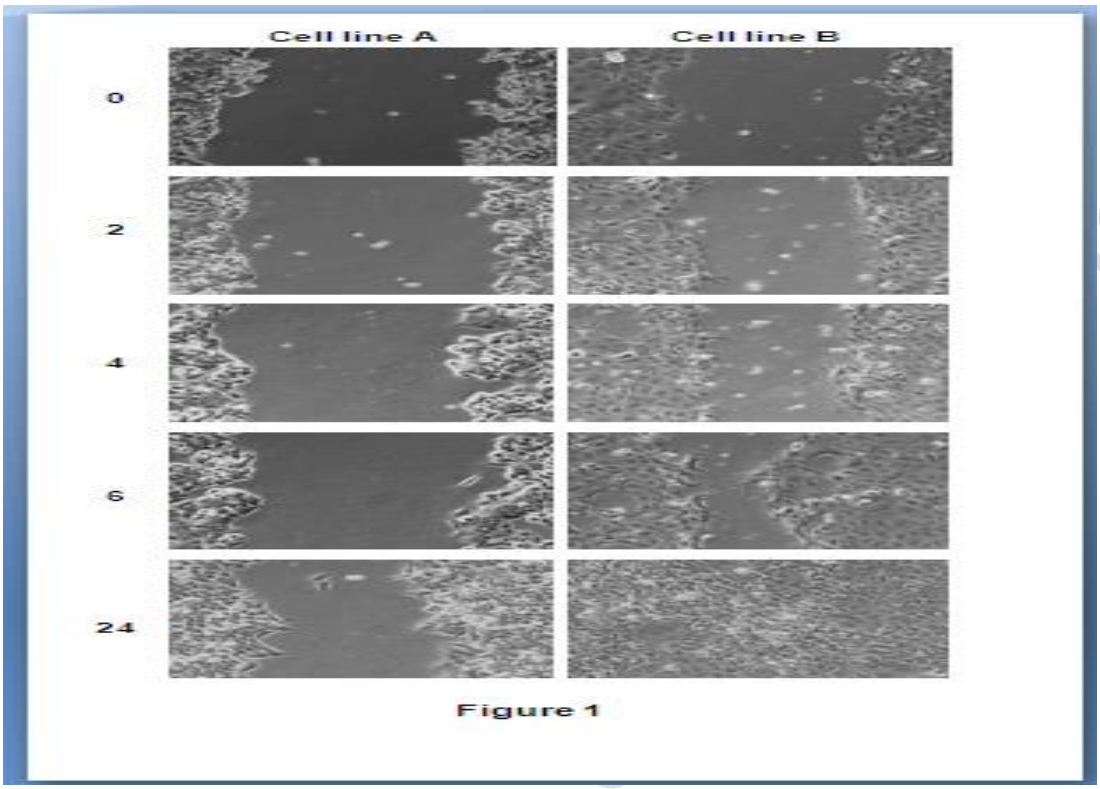
According to Cairns et al. (2011), multiple molecular mechanisms govern the intrinsic and extrinsic coverage of cancer cell metabolism to increase rapid ATP generation, increase in biosynthesis of macromolecules and a tightened maintenance of cellular redox status. Chambers et al. (2002) indicate that the growth of cancer cells is attributed to the Warburg effect, where the ATP generation is shifted from oxidative phosphorylation to glycolysis.

What aspects of experimental design influenced the outcomes you measured? How do these aspects qualify your interpretations? Do you see any changes in cell behaviour between the two different cell lines? What are your conclusions?

According to Riahi et al. (2012), the cell characteristics like the scraping speed and geometry can impact the results. According to Liang et al. (2007), migration of cells in a scratch assay can be impacted by both temperature and CO2 concentration of the incubator. The authors argue that the lack of effective temperature and CO2 content can lead to an 8-18 hour delay in cell migration to the scratch. Furthermore, the authors also contend that the lack of effective experimental conditions may result in the need for more cells to be seeded for analysis.

There is greater migration of cell line B when compared to cell line A. Since cell line B is tumorigenic, it can be contended that this cell line promotes the adhesion and spreading across the walls of the blood vessel due to the action of the actin cytoskeleton. However, the migration rate is relatively slower in cell line B, which may account for its slow invasiveness, as observed from following Figure 3.

Figure 3: Scratching Cells Assay Diagram



Cell lines A and B were subjected to invitro scratch assay at 0, 2, 4, 6 and 24 hours. The rate of migration can be determined by quantifying the number of cells which move from the edge of the scratch to the centre of the scratch. It is observed that while movement is observed in cell line A at 0, 2, 4 and 6 hours, there is limited movement at 24 hours. In contrast, a greater number of cells is found to move from the edge of the scratch for cell line B at all the time intervals, including 24 hours.

Having a quick search on the literature and the characteristics of cancer cells, what kind of suggestions could you make to start to explain what is happening in the cells and how it affects their growth?

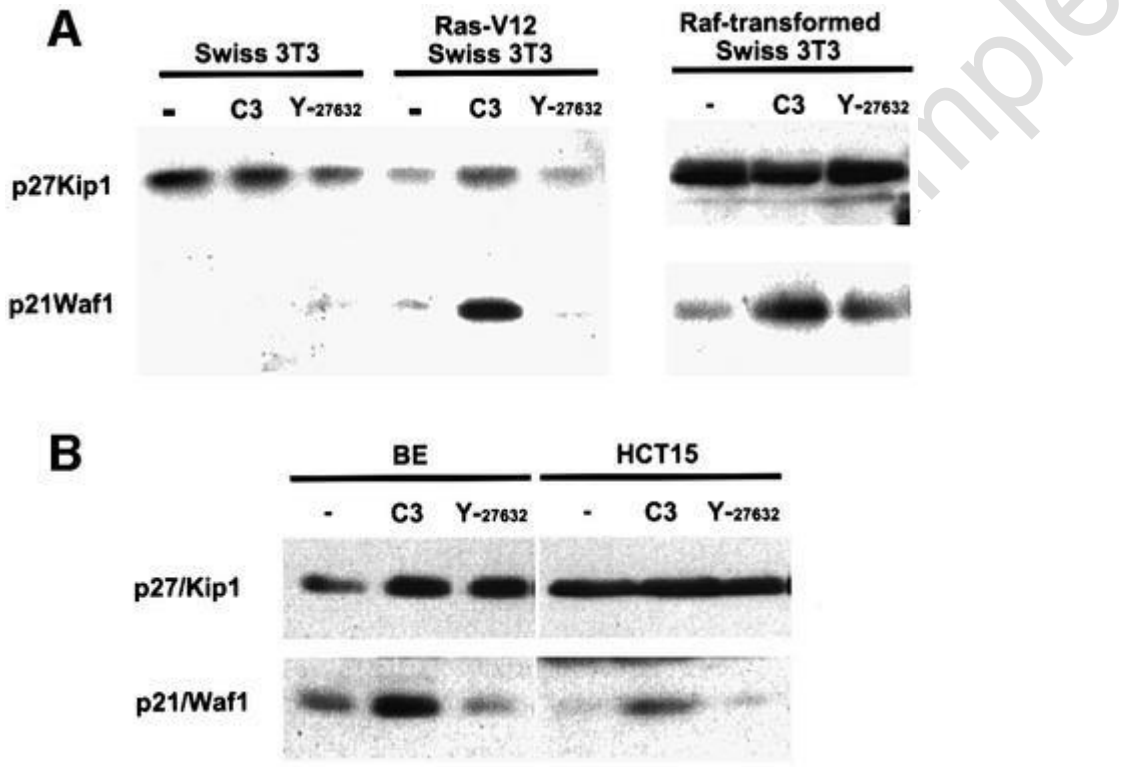
According to Friedl and Wolf (2003), the process of tumour-cell invasion and metastasis involves the migration of individual cells which can move from the tumour mass and migrate through the body using the lymphatic vessels or the bloodstream. The migration of cancer cells is regulated by the presence of matrix degrading enzymes, cell-to-cell communication and integrins. Some pro-migratory factors include chemokines, growth factors and partially degraded collagen (Sahai, 2005). These factors therefore are found to influence the growth of the cancer cell and its migration.

Any experiments you wish to suggest to refine the experiments?

1. DNA synthesis cell proliferation assay
2. Metabolic cell proliferation assay
3. Transmigration assay

Part B

This section discusses the results of Figure 3 of Sahai (2001) by providing the aim, method, results and conclusion.



Question: The question in this section asks to determine if there regulation of p21/Waf1 by Rho-GTP in cells which are Ras-transformed. The method used for final result determination is the western blotting. The aim of the results is to determine if Rho is found to regulate the levels of p21/Waf1 in the Ras-transformed cells as well as the colon carcinoma cells (parent cells).

Method: The technique of western blotting, also called an immunoblot, is most commonly used in biochemistry and molecular biology in order to identify the presence of specific proteins within a given sample tissue homogenate or from a sample extract. This method uses the principle of gel electrophoresis, wherein

the native proteins (either structured or denatured) are separated from one another by using the length of their polypeptide. The transfer of the protein to a nitrocellulose membrane is followed by treatment of the membrane with specific antibodies. The above Figures A and B provide this nitrocellulose membrane picture. In the current study, the use of the antibody for p27/Kip1 and p21/Waf1 is promoted. Depending on the concentration of the target protein, the blot can be visualised on the membrane.

Results: Panel A provides the results of the expression of p27/kip1 and p21/Waf1 for Swiss 3T3 cells, Ras-V12 Swiss T3 cell and Ras-transformed Swiss T3 cells which were seeded at 100,000 cells/3.5 cm dish. Tat C3, an expression construct for the Ce3 toxin, will inactivate RhoA and will therefore block the proliferation of Ras-transformed cells and inactivate RhoA. Similarly, Y-27632 is another pharmacological inhibitor which inhibits the Rho effector ROCK/Rho-kinase. From the results it is clearly observed that, while parental cells did not show expression of p21/Waf1, Ras-transformed cells were found to show slightly higher amounts of the cell cycle regulators. However, it is observed that the treatment of cells with ROCK/Rho-kinase inhibitor has limited impact on the p21/Waf1 levels.

Figure B, on the other hand, provides information on similar tests conducted with regard to BE cells and TCT 15 human colon carcinoma cell lines. This is carried out because these cell lines are considered to contain mutations which are oncogenic in nature (k-ras mutations). Results observed were similar to those observed with the Ras-transformed cells in terms of inhibition of Rho. Similarly, no results were obtained for ROCK/Rho-kinase. However, it is observed that the elevated levels of p21/Waf1 were high in these cell lines. Furthermore, it is observed that the level of p27/Kip1 was found to be high as a result of C3 and Y-27632 treatment in BE cells only. The HCT15 cells did not show any such change.

Conclusion: The results of the western blotting analysis clearly show that those cells which are transformed and contain oncogenic Ras are found to reduce the p21/Waf1 to levels which enable the growth of the cells.

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