Original Research Article

Effect of Growth Hormone on Cellular Transformation

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Abstract

Background: Hypermutability of Bloom Syndrome (BSyn) cells is the basis for this experimentation. Mutation of the *BLM* gene that encodes BLM protein results in defects of the DNA helices. Human growth hormone (HGH) has mitogenic effects that can cause an increase in the rate of mutation, which results in an increased risk of developing cancer.

Aim: The objective of this study is to observe the effects of hypermutability in relation to the growth and proliferation of cancerous cells.

Materials and Methods: Doxorubicin hydrochloride, a DNA damaging agent was used on both unaffected cell lines and those affected with Bloom Syndrome. Both groups were subsequently treated with growth hormone solutions of 10 ng/mL, 0.1 ng/mL, 0.001 ng/mL HGH. The soft agar colony formation was used to observe cell transformation. The top layer was .7% agarose and 1:1 complete culture medium, and the bottom layer was 0.5% agar and stained with .005% crystal violet. The cell lines were observed under the dissecting microscopeto observe the genomic instability of the cells, which has been defined as a mechanism for mutation.

Results: The Bloom syndrome cell line showed more sensitivity to treatments as opposed to the unaffected cell line. BSyn cells grown in the presence of 50nM DOX and 0.1 and 1.0ng/ml human growth hormone (HGH) show an increase in the number of colonies growing in soft agar.

Conclusion: Bloom syndrome cells exhibited genomic instability because of the missing functional BLM protein. They are hypersensitive to the DNA-damaging agent doxorubicin hydrochloride (DOX) as compared to cells with functional BLM protein.

Key words

Bloom Syndrome, BLM gene, Human growth hormone (HGH), DNA, Mutation.

Introduction

The autosomal recessive genetic disorder Bloom Syndrome, or BSyn, occurs after the biallelic pathogenic variation of the functional BLM gene which is cytogenetically located at 15q26.1, on the long (q) arm of chromosome 15 at position 26.1 [1]. The clinical picture of BSyn affected individuals shows a severe pre and postnatal growth deficiency, sparseness of subcutaneous fat tissue throughout infancy and early childhood, short stature throughout postnatal life, and an erythematous and sun-sensitive skin lesion of the face [2]. In this study, the hypermutability of these affected cells served as the central focus. We utilized doxorubicin hydrochloride (DOX) on a cell line affected with Bloom Syndrome, named HG2522, as well as on an unaffected cell line, that was noted to be "normal," HG2855. The use of the DNA damaging agent DOX, chemically defined as C27H29NO11, would test the hypothesis made on the effects of human growth hormone (HGH) on the genomic instability of the cells [3]. HGH This hormone may otherwise be known as somatotropin, C39H60N8O13, and is created by the body to stimulate cell growth; it serves the purpose of moving cells into the mitotic phase in this experiment [4]. The instability in the affected cell line has been noted as a mutation mechanism, suggesting that the Bloom Syndrome cells would be altered at a higher rate than those without which has been defined as a mechanism for mutation, presuming that those with Bloom Syndrome would transform and alter at a higher rate. The *BLM* gene provides instructions for making a member of a protein family called RecQ helicases. Because RecQ helicases help maintain the structure and integrity of DNA, they are known as the "caretakers of the genome [5]." More than 70 *BLM* gene mutations have been identified in people with Bloom syndrome [6]. BLM's accompanying protein is an ATP-dependent DNA helicase that unwinds single- and double-stranded DNA in a 3'-5' direction. This protein participates in DNA replication and repair, as it is involved in 5'-end resection of DNA during double-strand break (DSB) repair. It unwinds DNA and recruits DNA2 which mediates the cleavage of 5'-ssDNA [7]. This scientific data aided us with the foundation on which we built our thesis and constructed this research project.

Materials and methods

Two SV-40 transformed fibroblast cell lines, (GM00637G and GM08505B) are publicly available from the National Institute of General Medical Sciences Human Genetic Mutant Repository of the Cornell Institute for Medical Research, were used in this study. Cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% heat inactivated fetal bovine serum, using standard mammalian tissue culture techniques. Cells were incubated at 37°C/5% CO_2. In the initial cell subculture trypan blue (.4% solution) was utilized, its vital stain properties permeate dead cells and have a refractive effect on living cells. It was determined that 20,000 cells would be plated. The cells were plated in a 5% agar solution and later treated with 50 nM DOX, a DNA damaging agent for 48 hours and incubated at 37°C/5% CO_2.

The soft agar colony formation was used to observe cell transformation. The top layer was .7% agarose and 1:1 complete culture medium, and the bottom layer was 0.5% agar. The bottom layer prevents the cells from attaching to the bottom of the petri dish and allows for the later observation of the cells in suspension. Prepared growth hormone solutions of 10 ng/mL, 0.1 ng/mL, 0.001 ng/mL were used, these concentrations were chosen to see the effect of low, moderate, and high concentration of growth hormone on cells. The cells were plated, and a cell count vial was made and stained with trypan blue. The clonogenicity assay was stained with

6% glutaraldehyde and .5% crystal violet which resulted in overstraining of the cells, and no longer allowed the results to be easily observed. Therefore, it was determined that .005% CV would be used for future experiments. The Nanodrop was used to analyze nucleic acid, and RNA was isolated using chloroform. The staining process was revised, and .005% crystal violet was used, this staining was successful, and the cell lines were observed under the dissecting microscope. The colonies were able to be observed, and therefore showed a change in the initial morphology of the cells, and both cell lines showed growth. An additional clonogenicity assay was set up once again using triplicate cultures with untreated: DOX, HGH, and DOX + HGH treatments.

Table - 1.0: Colony Formation Assay.

Treatment Variables	No. of Colonies GM08505	No. of Colonies GM00637
0.01 nanograms/ml of HGH + 50 nanomolar DOX	1. 0 2. 4 3. 1 — 1.6	1. 4 2. 15 3. 7 — 8.6
0.1 nanograms/ml of HGH + 50 nanomolar DOX	1. 9 2. 3 3. 18 — 10	1. 3 2. 9 3. 0 — 4
1 nanogram/ml of HGH + 50 nanomolar DOX	1. 8 2. Too numerous 3. 32 — 20	1. 6 2. 11 3. 4 — 7
0.01 nanograms/ml of HGH + 75 nanomolar DOX	1. 5 2. 0 3. 2 — 2.3	1. 2 2. 2 3. 0 — 1.3
0.1 nanograms/ml of HGH + 75 nanomolar DOX	1. 9 2. Too numerous 3. 3 — 4	1. 4 2. 8 3. 4 — 5.3
1 nanogram/ml of HGH + 75 nanomolar DOX	1. 0 2. 0 3. 0	1. 1 2. 6 3. 1 — 2.6

Table 1.0:1,2,3 denote each of the three plates per treatment variable.
Blue denotes the average of all three plates per treatment variable.

Results

The hypothesis was that cells with Bloom syndrome would exhibit a high rate of cell transformation because of genomic instability, and therefore would provide merit to the conclusion that HGH hormone is a contradictory treatment practice. The results showed that HGH and DOX respectively have a significant effect on the rate of transformation of the cells in comparison to the untreated group. However, the combination of both HGH and DOX have an increased effect on transformation. The unaffected cell line also showed cell transformation at a slightly higher rate than that of the Bloom syndrome. However, in this case quantitative data was difficult to obtain as some plates had too many cells to count or the agar was broken and therefore could not be considered for counting.

We observed three different areas of our assay, and recorded the number of colonies that grew, in addition to recording the average number of colonies (**Table - 1.0:** Colony Formation Assay) We repeated the experiment twice, and chose the concentration based on results from the first experiment. It was decided that for trial two, we would use 75 Nm DOX. We determined that the affected cell line was hypermutable, and that the increased growth from HGH and increased DOX

resulted in less growth than the normal cell line, although the normal cell lines did not show a substantial difference in growth. There was a spike in growth in some of the Bloom cells, however the unaffected cells have more consistent values. In addition, it can be noted that although there is less growth in some of the plates, the DNA repair pathways are still functional in the normal cell line.

Table - 2.0: Colony Formation Assay Graph.

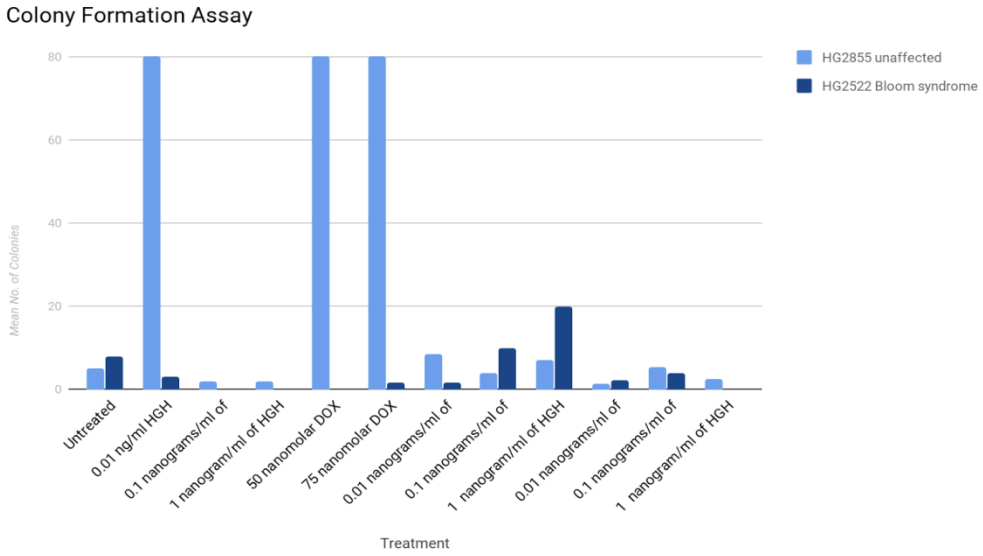
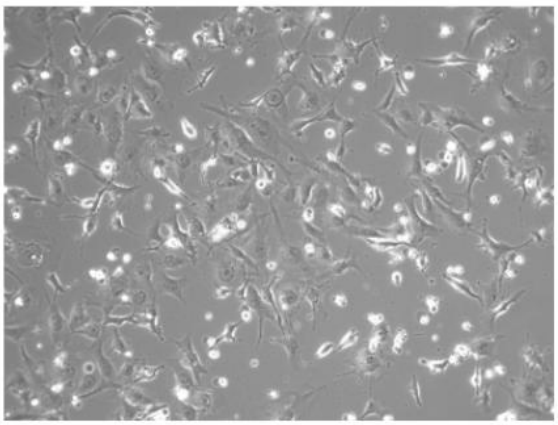
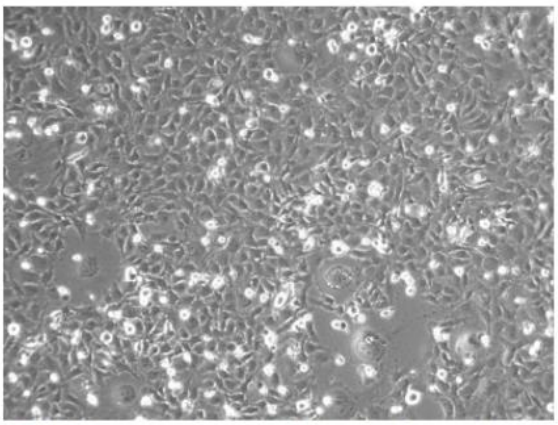


Table - 2.0 is the colony formation assay graph, in which the light blue indicates the unaffected cell line and the dark blue are cells with Bloom Syndrome. For charting purposes, treatments which resulted in numerous colonies were denoted as being >80, and 50 nanomolar DOX had significantly broken agar which was denoted as 0.

Figure - 1.0: 50 nm DOX and 1ng/ml HGH Comparison.



GM08505 treated for 48 hours with 50 DOX and 1.0ng/ml HGH. Highly refractile golden spheres are mitotic cells. Less cell growth compared to unaffected cultures.



GM00637 treated for 48 hours with 50 DOX and 1.0ng/ml HGH. Highly refractile golden spheres are mitotic cells. More growth is seen in cultures of unaffected cells.

Here are two photos taken from the dissecting microscope of both cell lines after being treated for 48 hours with 50 nm DOX and 1 ng/ml of HGH. The highly refractile gold cells, shown as white circles in these images, are mitotic cells. There is less cell growth on the left when compared to the unaffected cell line on the right. The BSyn cells are more damaged/ sensitive because they are unstable, and this was our expected result.

The bar graph (**Table - 2.0:** Colony Formation Assay) illustrates our untreated controls, along with different combinations of HGH and DOX on our cell line (.1ng HGH and 50 nM Dox, 1 ng/ml HGH and 50 nM, .01 HGH and 75 nM Dox).The Bloom cell line is the darker blue and shows a greater number of transformed colonies as compared to normal. An important result to note is that .01ng HGH with 75 nM DOX shows a large decrease in the number of BSyn colonies compared to the first round with 50 nM DOX. Although the graph may at first seem inconsistent for cultures with higher treatment concentrations, we determined that it may be explained as a hypersensitivity of Bsyn cells to the DOX, increasing the growth and leading to cell death at a faster rate. In fact, phase micrographs of the cell cultures taken on the day cells were plated in soft agar indicated that there was decreased cell growth and increased numbers of dead cells in these cultures.

Photos of the cells for comparison purposes (**Figure - 1.0:** 50 nm DOX and 1ng/ml HGH Comparison), the Bloom cells appear to be more damaged, because of their instability and sensitivity. This was directly in line with our proposed hypothesis.

Discussion

Clinical research has shown that Bloom syndrome cancers respond well to DOX treatment [8]. The conclusion of the experimentation shows that Bloom syndrome cells were significantly decreased in the presence of DOX, which is in alignment with the literature. Further research would be facilitated by repeating the experiment in continuous cultures over time to confirm the results obtained. In addition, evaluating gene expression analyses performed with RNA extracted cells from parallel experiments to see if the expression of genes involved in DNA repair are elevated in the experimental group. Lastly, gene expression analyses of genes involved in the Growth Hormone/Insulin-like Growth Factor Hormone axis may reveal some association with increased tumorigenesis in Bloom syndrome, and we can look further into the correlation of this aspect [9].

Conclusion

In conclusion, we determined that the affected cell line showed more sensitivity to treatments as opposed to the unaffected cell line. Bloom syndrome cells exhibit genomic instability because they are missing functional BLM protein. They are hypersensitive to the DNA-damaging agent doxorubicin hydrochloride (DOX) as compared to cells with functional BLM protein. BSyn cells grown in the presence of 50nM DOX and 0.1 and 1.0ng/ml human growth hormone (HGH) show an increase in the number of colonies growing in soft agar.

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