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Evaluating Homogeny of Cellular Mitotic Cytosolic Protein Antigens of Chinese Hamster Ovary (CHO), HeLa, Vero and Human Lymphocytes

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Abstract: Mitotic factors peak at the G2-mitotic transition and have no species specificity. Altered cell cycle dynamics are hallmarks of cancers and this leads us to believe in altered protein profiles in cancerous conditions distinct from normal cells. Antiserum raised against CHO cytosolic mitotic protein antigens was tested with cytosolic interphase and mitotic protein extracts of HeLa, Vero and normal human lymphocytes. The mitotic and interphase extracts of transformed cells showed distinct reactions with the antibodies. Constitutively expressed proteins, particularly, those in the higher molecular weight range presumably contribute to the cross reactivity. Neither mitotic nor interphase extracts of normal human lymphocytes reacted with the antiserum, indicating the homogeneity of cytosolic mitotic protein antigens among cancerous cell lines and their shared epitope matching. The possible applications of this are in areas including cancer immunotherapy; for the detection and selective destruction of such cells in a population without disturbing normal cell functions.

Key words: Cancerous cells, mitotic extracts, antibodies, cross-reactivity

Introduction

The information available on mitotic factors is from studies related to the phenomenon of premature chromosome condensation. Normally the chromosomes of eukaryotic cells are visible only for a brief period during cell division. However, when mitotic cells are fused with interphase cells, factors present in the mitotic cytoplasm induce breakdown of the nucleus and reorganization of the interphase chromatin into Prematurely Condensed Chromosomes (PCC). The mitotic factors have been found to migrate to the interphase nucleus and subsequently become associated with the prematurely condensed chromosomes (Sperling and Rao, 1974). Course of accumulation of mitotic factors during the cell cycle is important for the regulation of the cell cycle. The mitotic factors accumulate slowly in the beginning of G2 but at a progressively more rapid rate during late G2 and reach a threshold at the G2-mitotic transition when the nuclear membrane breaks down and chromatin condenses into chromosomes (Sunkara *et al*., 1979).

The fact that mitotic factors have no species specificity has been established and it was demonstrated that mitotic cells of human origin (HeLa), upon fusion, can induce PCC in cells from a variety of animal species including mammals, birds, amphibians, fishes and insects and mitotic cells from these species can induce PCC in HeLa cells indicates that the factors involved in the induction of this phenomenon are common over a wide range of animal species. In fact plant cells have been shown to induce PCC in mammalian cells. The factors involved are most likely proteins since, if the mitotic inducer cells are prelabeled with radioactively tagged aminoacids, labeled protein can be

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observed to be transferred from the mitotic component to the interphase chromosomes (Hittelman, 1986; Rao, 1982). Because meiotic maturation appears to be similar to the induction of PCC, particularly with regard to breakdown of the nuclear membrane and condensation of chromosomes, studies were carried out to test whether mitotic factors from mammalian cells could induce maturation in amphibian oocytes and the results suggested that mitotic factors from mammalian cells can induce Germinal Vesicle Break Down (GVBD) and chromosome condensation in amphibian oocytes and these factors have no species barriers and also the factors involved in the breakdown of nuclear membrane and chromosome condensation, which are associated with three different phenomenonviz., mitosis, meiosis and PCC-appear to be very similar, if not identical, throughout the animal kingdom.

Cancerous cells are different from normal ones by their altered cell cycle dynamics. This might also lead us to believe in altered protein profiles in cancerous conditions, which might not be apparent in cells with normal cell cycles. The common factor in cancers of any cell type and also in most of the available continuous culture cell lines is the misregulated cell cycle checkpoints. Presuming that mitotic factors are evolutionarily conserved and hence have no cross-species boundaries, these might be of use to develop common detection or interventional tools. Therefore three established cell lines of different phylogenetic origins, CHO, Vero and HeLa were selected for the present study.

By virtue of the affinity to antigenic epitopes, antibodies prove to be useful tools for evaluating homogeneity of mitotic cytosolic protein antigens. An important point of consideration can be the cross reactivity of the antibodies to structurally similar antigenic epitopes which might prove to be valuable in comparing soluble proteins.

Materials and Methods

CHO (Chinese hamster ovary) cell lines were cultured in Dulbecco's modified Eagle's medium containing 10% FBS at 37°C in humidified atmosphere containing 5% CO_2. Cells were synchronized at mitotic phase by addition of 75 µL of colcemid and incubated for 2 h. Maximum number of mitotic cells was harvested by selective detachment. Mitotic cytosolic proteins were extracted using Tris based reagent; which extracted only the most soluble proteins, the cytosolic proteins. This was achieved by the washed cell-pellet being mechanically agitated using Teflon coated pestle followed by centrifugation at 1000 rpm for 10 min. The supernatant containing the proteins was aspirated and a buffer exchange was performed by dialysis with 1X Phosphate Buffered Saline (PBS). The proteins extracted from mitotic cells were processed and purified by sephadex gel filtration using Phosphate Buffered Saline as the wash buffer. The concentration of the proteins in the whole cell mitotic extract following purification by gel filtration was estimated using Bradford protein Assay.

One healthy previously unimmunised rabbit was given a subcutaneous injection of 1 mL sterile soluble mitotic extract (filtered through 0.22 µ filter) emulsified with an equal volume of complete freund's adjuvant at multiple sites. The rabbit was given two boosters of 1 mL (emulsified with incomplete freund's adjuvant) on 17th and 25th day after first immunization. Blood samples were collected on the 7th, 24th and 32nd day after the first immunization, allowed to clot at room temperature and the antiserum collected. The antiserum thus collected was tested by countercurrent electrophoresis to confirm the presence of antibodies for CHO mitotic extract. The titer of the antiserum was established by conventional Agarose double diffusion. Purification of IgG from whole serum was done by Proteinase A column with Phosphate Buffered Saline as the buffer. Thus, three sets of reactants; whole serum, serum devoid of IgG and purified IgG were obtained and were individually tested for reactivity with CHO mitotic extracts.

Mitotic cells of HeLa, Vero, CHO and human lymphocytes were harvested by established culture methods. These were used to extract mitotic cytosolic proteins and interphase cells were collected by trypsinization of the cell lines and by simple density gradient separation of human venous blood to check for reactivity of the anti CHO-mitotic cytosolic protein antigens. This was achieved by double diffusion in 1.0% Agarose.

Table 1: Reactivity by precipitation of the anti CHO-mitotic cytosolic protein polyclonal antibodies demonstrating the homogeneity of the mitotic cytosolic protein antigens across a few species-boundaries in cancerous condition

Antigen	Antibody	Reactivity by precipitation
CHO mitotic cytosolic proteins	Anti whole serum	Positive
CHO mitotic cytosolic proteins	Anti whole serum devoid of IgG	Negative
CHO mitotic cytosolic proteins	Purified IgG	Positive
HeLa mitotic cytosolic proteins	Anti whole serum	Positive
HeLa mitotic cytosolic proteins	Anti whole serum devoid of IgG	Negative
HeLa mitotic cytosolic proteins	Purified IgG	Positive
Vero mitotic cytosolic proteins	Anti whole serum	Positive
Vero mitotic cytosolic proteins	Anti whole serum devoid of IgG	Negative
Vero mitotic cytosolic proteins	Purified IgG	Positive
Normal Human lymphocyte cytosolic proteins	Anti whole serum	Negative
Normal Human lymphocyte cytosolic proteins	Anti whole serum devoid of IgG	Negative
Normal Human lymphocyte cytosolic proteins	Purified IgG	Negative

Results

Serum isolated following immunization showed presence of antibodies for whole cell CHO mitotic extract and its titer was established as 1:8 after the second booster. The IgG fraction isolated by affinity chromatography tested by immunodiffusion for its contribution to the reactivity of the antiserum showed distinct precipitation after concentration by solid sucrose dialysis on comparison with the same before concentration (in which reaction was absent owing to its highly diluted condition). The serum devoid of IgG did not show reactivity thus indicating a secondary antibody response containing predominantly IgG.

The mitotic extracts of CHO, HeLa and Vero showed distinct precipitin reactions with the Whole serum and the IgG fraction of the antiserum. It was also noticed that the antiserum showed distinct precipitin reactions with the interphase extracts of the above mentioned cell lines. This is clearly due to the protein similarities in the mitotic and interphase stages of the cell lines as was observed by SDS PAGE analysis. Although, there were distinct proteins in the mitotic extracts, predominantly constitutively expressed proteins, especially in the higher molecular weight range were noticed. This explains the cross-reactivity of the antiserum to both interphase and mitotic extracts of the three cell lines. Interestingly, neither the mitotic nor the interphase extract of the non-cancerous (normal) human lymphocytes showed reactivity with the antiserum developed (Table 1).

Discussion

In the present study the reactivity of the Rabbit Anti CHO mitotic extract with cytosolic proteins of mitotic and interphase cells of CHO, Vero, HeLa and normal human lymphocytes was studied to evaluate the homogeneity of the cytosolic protein antigens. The results demonstrate the reactivity of the whole serum and IgG fraction to the mitotic extracts from cancerous cell lines (CHO, Vero and HeLa) and failed to react with the protein extracts from normal human lymphocytes indicating the presence of unique and evolutionarily conserved proteins with matching epitopes in cancerous cells, which are not present in normal cells (human lymphocytes in this study). It can also be inferred that the reactivity of the antiserum is strongly influenced by the presence of these shared epitope identities. The extract from normal human lymphocytes, which seemed to lack the epitope match, was unable to react with the antiserum.

Conclusions

The experimental results in evaluation of homogeneity of cytosolic mitotic and interphase protein antigens among cancerous cell lines and normal cells (human lymphocytes) revealed the preferential reactivity with cytosolic protein antigens of cancerous cell types.

A cross-reactivity of the antibodies to mitotic cancerous cells when compared to normal interphase cells was observed. This indicates the homogeneity of cytosolic mitotic protein antigens among cancerous cell lines and their shared epitope matching. This is augmented by the fact that, polyclonal antibodies raised against the cytosolic protein antigens from one cancerous cell type can be used in applications related to other similar transformed cell types. This might lead to the possible application of the polyclonal anti mitotic extract antibodies in areas such as cancer immunotherapy; for the detection and possible selective destruction of such cells in a population without disturbing the normal cell functions.

References

Hittelman, W.N., 1986. The technique of premature chromosome condensation to study the leukemic process: Review and speculations. Crit. Rev. Oncol. Hematol., 6: 147-221.

Rao, P.N., 1982. The Phenomenon of Premature Chromosome Condensation. London: Academic Press Inc. Ltd., pp: 2-40.

Sperling, K. and P.N. Rao, 1974. The phenomenon of premature chromosome condensation: Its relevance to basic and applied research. Humangenetik, 23: 235-258.

Sunkara, P.S., D.A. Wright and P.N. Rao, 1979. Mitotic factors from mammalian cells induce germinal vesicle breakdown and chromosome condensation in amphibian oocytes. Proc. Natl. Acad. Sci., 76: 2799-2802.