Vol. 185, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1992 Pages 271-276

MOUSE AND HUMAN HEMOPOIETIC CELL LINES OF ERYTHROID LINEAGE EXPRESS LAMINS A,B AND C

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Received April 13, 1992

Using monoclonal antibodies, we have studied the expression of lamins A,B,C and vimentin in mouse and human erythroleukemia cells . We have found that in contrast with previous reports these cells have all three lamins. Mouse cells lack vimentin, whereas human cells express it. Lamins B and C are the most abundant lamins, whereas considerably less lamin A is detectable. Our results argue that some mouse and human hemopoietic cells can express all three lamins and that production of vimentin does not necessarily precede that of lamins A/C, as other reports have suggested in the past. The data also show that the absence of a salt resistant inner nuclear matrix is not always related with the lack of lamins A/C and vimentin, as recently proposed.

The nuclear lamina is a fibrous meshwork located underneath the inner nuclear membrane (1-2).The main constituents of the lamina are lamins A,B and C, polypeptides with molecular weights between 70 and 62 kDa , although additional minor components have been identified (3).Lamin B is connected to the nuclear membrane by means of its interaction with a specific integral protein (4), whilst lamins A/C are DNA-binding proteins (5-6).While lamin B is expressed throughout the development, lamins A/C are absent from several embryonic tissues and they cannot also be detected in cells from immune and hemopoietic systems (7-12).It is generally thought that the expression of lamins A/C is closely related to that of intermediate filament proteins such as vimentin, with which lamins A/C share sequence homologies (13). In this report we show that mouse and human erythroleukemia cell lines express all three lamins. We also demonstrate that while mouse cells do not produce any detectable vimentin, human cells express such a protein.

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0006-291X/92 $4.00

MATERIALS AND METHODS

Source of antibodies Monoclonal antibody B6 (recognizing lamins A/C) and monoclonal antibody J16 (recognizing lamins A,B,C) were a kind gift by Dr. R. Berezney, Dept. of Biological Sciences, State University of New York at Buffalo, Buffalo, New York, USA. The monoclonal anti-vimentin antibody VIM-13.2 was obtained from Sigma Chemical Company, St. Louis, MO, USA. All three monoclonals are of IgM subclass. J16 was used diluted 1:50, B6 diluted 1:200 (both as supernatant from the culture) while VIM-13.2 was employed diluted 1:100 (as ascitic fluid).

Cell culture Swiss 3T3 mouse fibroblasts (from Dr. K. Brown, Insitute of Animal Physiology and Genetics Research, AFRC, Cambridge, UK) were grown in Dulbecco's modified minimum essential medium supplemented with 10% newborn calf serum. Mouse erythroleukemia cells (clone 707, Friend cells) and human erythroleukemia cells K562 were grown in RPMI-1640 medium supplemented with 10% fetal calf serum. The mouse cell line was originally provided to us by Istituto Superiore di Sanità, Roma, Italy and it has been extensively used in our laboratory for erythroid differentiation-related studies (14-15). K562 cells were obtained from the American Type Culture Collection (CCL 243).

Preparation of subcellular fractions Total cell proteins were obtained by directly dissolving cells in boiling Laemmli's sample buffer (16) supplemented with 6M urea. The suspension was passed several times through a 25 g needle to shear chromosomal DNA and reduce viscosity. The nuclear matrix fraction was prepared from mouse and human erythroleukemia cells as previously indicated (17) and then dissolved in sample buffer as above.

One and two-dimensional polyacrylamide gel electrophoresis For one dimensional analysis, proteins were separated on a 8% polyacrylamide, 0.1% sodium dodecyl sulfate (SDS) according to Laemmli (16). For two-dimensional separations the non equilibrium pH gradient electrophoresis technique (2-D NEPHGE), described by O'Farrell et al. (18). The second dimension gel was a 8% polyacrylamide, 0.1% SDS gel. When required, gels were stained with 0.125% Coomassie Brilliant Blue R-250 dissolved in 50% methanol-10% acetic acid.

Western blotting Proteins from one and two-dimensional gels were electrophoretically transferred to nitrocellulose paper as previously indicated (19). Immunological detection of antigens was according to Martelli et al. (20). Alkaline phosphatase-conjugated anti mouse IgM (diluted 1:500, Sigma Chemical Company) were used to detect primary antibody binding.

RESULTS AND DISCUSSION

In Fig. 1 we show the results from one dimensional Western blotting experiments using VIM-13.2 antibody. It is evident that mouse erythroleukemia cells do not contain any detectable vimentin, which was nevertheless recognized as a single band at 57 kDa among proteins prepared from both mouse fibroblasts and human K562 cells.

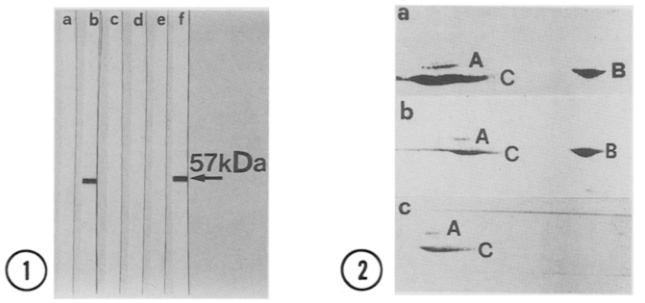

Figure 1. One dimensional Western blotting using anti-vimentin antibody VIM-13.2. Whole proteins from mouse 3T3 cells (a,b), mouse Friend cells (c,d) and human K562 cells (e,f) were separated on SDS-polyacrylamide gels and blotted to nitrocellulose paper which was then probed with monoclonal antibody VIM-13.2. Protein prepared from an equivalent number of cells for each sample was blotted to each strip. b,d,f: secondary antibody only.

Figure 2. Two-dimensional Western blotting using monoclonal antibodies J16 and B6. Nuclear matrix proteins from mouse Friend cells (a,c) and human K562 (b) were separated by two-dimensional polyacrylamide gel electrophoresis. In the first dimension migration was from right (acidic) to left (basic). Bio-Lytes ampholytes pH 3,5 - 10 (Bio-Rab Lab., Richmond, CA, USA) were employed. Blotted proteins were then probed with monoclonal antibody J16 (a,b) which recognizes lamins A, B, C and B6 (c) which recognizes lamins A/C. The calculated molecular weight of the spots is 70 kDa (lamin A), 66 kDa (lamin B) and 62 kDa (lamin C).

We next prepared from Friend and K562 erythroleukemia cells the nuclear matrix fraction, i.e. the nuclear fraction which resists nuclease treatment and high salt (2M NaCl) extraction and that contains also the peripheral lamina (21). Proteins from nuclear matrix were separated by two-dimensional gel electrophoresis, transferred to nitrocellulose which was then probed with monoclonals J16 and B6. The results from these experiments are shown in Fig. 2a,b and c.

J16 (which recognizes all three the lamins) stained 3 spots in blots from mouse and human erythroleukemia cells whereas B6 (which recognizes lamins A/C) stained only two spots in blots from Friend cells. We did not test B6 against matrix proteins from K562 cells. It is evident from these experiments that only small amounts of lamin A are present in the two cell lines whereas lamins B and C are more abundant. Coomassie Blue stained two-dimensional gels of nuclear matrix proteins are presented in Fig. 3a and b. The position of lamins A,B,C corresponds to that obtained in Western blotting experiments with monoclonal antibody J16. From this picture it is easy to see that even in dye-stained gels lamins B and C are more represented than lamin A. Both lamin A and C appear as elongated spots, conceivably constituted from several isoforms. Similar results were obtained if Friend cells were induced to differentiate into globin-producing elements by treatment for 4 days with dimethylsulfoxide (not shown).

To our knowledge this is the first report dealing with the presence of lamins A/C in undifferentiated mouse and human hemopoietic cell lines. Indeed previous studies have shown that several mouse cell lines of hemopoietic and immune origin lack

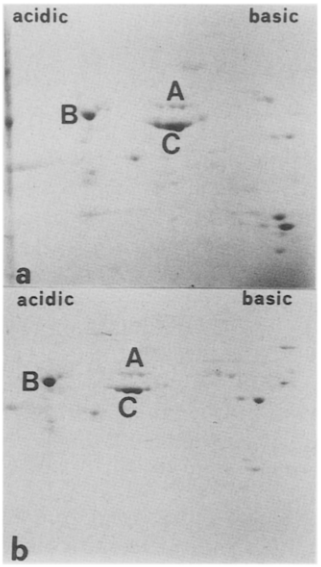

Figure 3. Coomassie Blue stained two-dimensional gels of nuclear matrix proteins from mouse Friend cells (a) and human K562 cells (b). In the first dimension migration was from left (acidic) to right (basic). The position of lamins A, B, C is indicated (A,B,C) as deduced from two-dimensional Western blots. 300 μg of protein was loaded in the first dimension. Protein was assayed according to Lowry et al. (30).

lamins A/C (11). Similar results have been obtained with human cell lines (10). In this sense it should be noted that in human and rat macrophagic cells, production of lamins A/C can be obtained upon treatment with thioglycollate (22). It should be pointed out that the cells we employed for these experiments are of erythroid lineage, being capable to differentiate into hemoglobin producing elements upon exposure to dimethylsulfoxide or tiazofurin (14,15,23). In the past only cell lines of lymphoid and myeloid lineage have been investigated to study expression of lamins A/C and this could explain the different results. However, the expression of lamins A/C in erythroid cells is generally thought to be characteristic of more mature elements, whereas ours are undifferentiated cells (11). Another distinctive feature of the two cell lines we studied is that little lamin A is present compared with lamin C. Usually, the levels of lamins A/C are similar in cells producing both of these proteins (24-26). This suggests that the production of lamins A/C can be differentially regulated even though they are thought to be products of the same gene (11). It is also worth mentioning that mouse cells do not express vimentin, while human K562 cells produce it. In general, cell lines expressing lamins A/C produce also vimentin, although there exists in the literature a single report concerning a human adrenocarcinoma cell line which express all three lamins but not vimentin (27). Thus, in contrast to suggestion of previous reports it may be possible that production of lamins A/C does not always precede expression of vimentin (11). Lastly, we would like to stress that our morphological investigations

carried out on the nuclear matrix fraction prepared from Friend cells have clearly established the absence of an internal structure which spontaneously resists high salt extraction (17). Indeed such an inner structure can be seen only after heat-induced "stabilization" of isolated nuclei, even if a mild extraction procedure (i.e. 0.2 M $(NH_4)_2SO_4$) is used (28). Similar results have been obtained with K562 cells (data not shown). Again we note that our results differ from the data of other investigators who have argued that the absence of a salt stable inner matrix is directly related with the lack of lamins A/C and vimentin (29). It is conceivable that such a clear cut difference is not applicable to all cell lines. We think that our data further demonstrate the extreme variability existing among different cell lines as far as the expression of lamins and intermediate filament proteins is concerned. Thus we conclude that before any definitive conclusion can be drawn further and deeper investigations are necessary in this field.

ACKNOWLEDGMENTS

The authors wish to thank Dr. R. Berezney for the gift of monoclonal antibodies and Mr. A. Fantazzini for the illustrations. This work was supported by Italian C.N.R. grants PF BTBS and PF IG.

REFERENCES

1. Krohne, G., Benavente, R.(1986) Exp. Cell Res. 162, 1-10.
2. Gerace,L., Burke, B. (1988) Annu. Rev. Cell Biol. 4, 335-374.
3. Kaufmann, S.H. (1989) J. Biol. Chem. 264, 13946-13955.
4. Worman, H.J., Yaun, J., Blobel, G., Georgatos, S.D. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 8531-8534.
5. Shoeman, R.L., Traub, P. (1990) J. Biol. Chem. 265, 9055-9061.
6. Hakes, D.J., Berezney, R. (1991) J. Biol. Chem. 266, 11131-11140.
7. Lebel, S., Lampron, C., Royal, A., Raymond, Y. (1987) J. Cell Biol. 105, 1099-1104.
8. Rober, R.-A., Weber, K., Osborn, M. (1989) Development 105, 365-378.
9. Pandey, S., Parnaik, V.K. (1991) Biochem. Biophys. Res. Commun. 179, 1082-1087.
10. Paulin-Levasseur, M., Scherbarth, A., Traub, U., Traub., P. (1988) Eur. J. Cell Biol. 47, 121-131.
11. Rober, R.-A., Sauter, H., Weber, K., Osborn, M. (1990) J. Cell Sci. 95, 587-598.
12. Guilly, M.-N., Kolb, J.-P., Gosti, F., Godeau, F., Courvalin, J.-C. (1990) Exp. Cell Res. 189, 145-147.
13. Fisher, D.Z., Chaudhary, N., Blobel, G. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 6450-6454.
14. Cocco, L., Gilmour, R.S., Maraldi, N.M., Martelli, A.M., Papa, S., Manzoli, F.A. (1985) Biol. Cell, 54, 49-56.
15. Cocco, L., Gilmour, R.S., Ognibene, A., Letcher, A.J., Manzoli, F.A., Irvine, R.F. (1987) Biochem. J. 248, 765-770.
16. Laemmli, U.K. (1970) Nature (London) 227, 680-685.

17. Martelli, A.M., Falcieri, E., Gobbi, P., Manzoli, L., Gilmour, R.S., Cocco, L. (1991) Exp. Cell. Res. 196, 216-225.
18. O'Farrell, P.Z., Goodman, H.M., O'Farrell, P.H. (1977) 12, 1133-1142.
19. Martelli, A.M., Carini, C., Marmiroli, S., Mazzoni, M., Barker, P.J., Gilmour, R.S., Capitani, S. (1991) Exp. Cell Res. 195, 255-262.
20. Martelli, A.M., Neri, L.M., Gilmour, R.S., Barker, P.J., Huskisson, N.S., Manzoli, F.A., Cocco,L. (1991) Biochem. Biophys. Res. Commun. 177, 480-487.
21. Verheijen, R., Van Venrooij, W., Ramaekers, F. (1988) J. Cell Sci. 90, 11-36.
22. Rober, R.-A., Gieseler, R.K., Peters, J.H., Weber, K., Osborn, M. (1990) Exp. Cell Res. 190, 185-194.
23. Olah, E., Natsumeda, Y., Ikegami, T., Kote, Z., Horanyi, M., Szelenyi, J., Paulik, E., Kremmer, T., Hollan, S.R., Sugar, J., Weber, G. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 6533-6537.
24. Kaufmann, S.H., Mabry, M., Jasti, R., Shaper, J.H. (1991) Cancer Res. 51, 581-586.
25. Kaufmann, S.H., Shaper, J.H. (1984) Exp. Cell Res. 155, 477-495.
26. Belgrader, P., Siegel, A.J., Berezney, R. (1991) J. Cell Sci. 98, 281-291.
27. Osborn, M., Weber, K. (1987) Exp. Cell Res. 170, 195-203.
28. Martelli , A.M., Falcieri, E., Gobbi, P., Manzoli, L., Cataldi, A., Rana, R., Cocco, L. (1992) Cell Biol. Int. Rep. (in press).
29. Wang, X., Traub, P. (1991) J. Cell Sci. 98, 107-122.
30. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.