Biophys Struct Mech (1982) 9: 89–93

Biophysics of Structure and Mechanism
© Springer-Verlag 1982

Intercellular Communication, Three-Dimensional Cell Contact and Radiosensitivity*

H. Dertinger[1], G. Hinz[1], and K. H. Jakobs[2]

[1] Kernforschungszentrum Karlsruhe, Institut für Genetik und für Toxikologie von Spaltstoffen, Postfach 36 40, D-7500 Karlsruhe 1, Federal Republic of Germany
[2] Pharmakologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, Federal Republic of Germany

Abstract. When electrically coupled mammalian cells are cultured as spherical clones (spheroids) and exposed to ionizing radiation they are less radiosensitive than monolayers of the same cell line. Investigations into the possible role of coupling (gap junctions) and three-dimensional contact in the expression of this phenomenon revealed
1) a correlation between cell coupling and the activity of adenylate cyclase in monolayers,
2) a sharp drop of cyclase activity in spheroids of coupled cells compared to monolayers, and
3) a decrease of coupling with age ("maturation") of the spheroids.

These results suggest profound physiological alterations in communicating cells induced under conditions of tight three-dimensional contact as a possible cause for the reduced radiosensitivity of spheroids.

Key words: Intercellular communication – Spheroids – Adenylate cyclase – Radiosensitivity

From recent results it is evident that intercellular communication (electrical coupling) may drastically alter the radiosensitivity of mammalian cells in vitro. When cells of the same line were cultured as conventional monolayers and as multicellular spheroids (spherical cell clones), the spheroid cells survived much better after γ-irradiation than the monolayer cells (Durand and Sutherland 1973; Dertinger and Hülser 1981). However, this was only observed with electrically coupled cells (Dertinger and Hülser 1981). Further characterization revealed that this increased radioresistance of coupled spheroid cells is independent of the proliferative status of the cells (Dertinger and Hülser 1981) and "protects" cells against various forms of cytogenetic damage (Hinz 1982). Moreover, this type of

* Based on material presented at the Symposium "Intercellular Communication" Stuttgart, September 16–17, 1982

0340-1057/82/0009/0089/$ 01.00

radioresistance was very recently identified also in human tumor xenografts (Guichard et al. 1982). Of particular interest is the fact that three-dimensional contact as encountered in the spheroids is required for the expression of this communication-dependent radioresistance. Here we present data which show that this phenomenon is probably linked to physiological alterations induced in coupled cells during spheroid culture.

Material and Methods

Spheroids can be grown from most cell lines adapted to form monolayers. When small aggregates of these cells are introduced into spinner culture they grow in size by multiplication of their outer cells. Unless otherwise specified, the spheroids used for the present experiments had a diameter of 270–300 μm (~ 10^7 μm^3 in volume) containing several thousand fully viable cells; 3–8 days of culture, depending on the cell line, are required to obtain spheroids of this size. The same culture medium (Eagle's MEM) and serum concentration (15% FCS) was used for spheroids and monolayers. Details of culture, irradiation and survival test (colony forming criterion) are given elsewhere (Dertinger and Hülser 1981). Cell-coupling was measured using micro-electrode techniques (Hülser and Webb 1973). Activity of membrane-bound adenylate cyclase was determined in broken cell preparations by the method of Jakobs et al. (1976) using [α-^{32}P]ATP as substrate.

Results and Discussion

Figure 1 gives an example of increased radioresistance of cells cultured and irradiated as spheroids. Evidently, the survival curve of the monolayer is much

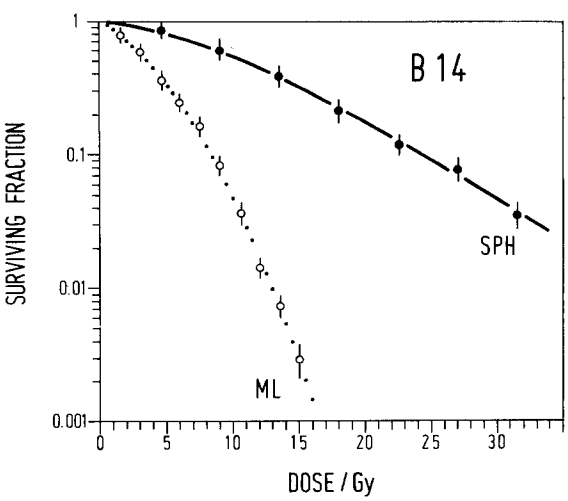

Fig. 1. Survival curves of Chinese hamster B14 FAF28 monolayer (*ML*) and spheroid (*SPH*) cells after Co-γ-irradiation. Survival is expressed as fraction of colony formers relative to unirradiated controls. Cultures were trypsinized immediately after irradiation and the cells tested for survival

Table 1. Activity of adenylate cyclase (pmol/mg protein/10 min)

	L	V79	3T3	B14	
Single cells	34.1	197.6	15.8	143.4	(11.9)
Exponential monolayers	38.9	132.2	9.0	69.4	(5.8)
Plateau monolayers	51.2	183.2	10.0	82.1	
Spheroids	53.4	91.1	6.9	44.3	(8.1)

Values represent averages of two independent experiments with three-fold determination per experiment. For further details see Jakobs et al. (1976) and Hinz (1982). In brackets: Electrical input resistances (MΩ) of B14 FAF28 cells in the individual cultures

steeper than that of the spheroids. The Chinese hamster B14 FAF28 cell line used was shown to be coupled (Dertinger and Hülser 1981).

It is tempting to relate the increased radioresistance of spheroids to intercellular exchange of small molecules (e.g., associated with DNA repair) during or shortly after irradiation ("helper function"). However, a closer examination reveals that this phenomenon rather reflects a single-cell property acquired by communication and growth of cells within the three-dimensional cellular matrix of the spheroids. When spheroids are trypsinized into single cells prior to irradiation, the enhanced resistance decays only slowly with time and can be detected even 6 h after trypsinization (results not shown). Moreover, if the net exchange of specific molecules would be the principal mechanism of the spheroid radioresistance one would expect this phenomenon to occur also in monolayers, which is not the case (Dertinger and Hülser 1981). Even the hypothesis that cell coupling is higher in spheroids thus allowing molecular exchange to proceed at a higher rate than in the monolayer cannot be maintained (see below!).

Since under certain conditions activators of adenylate cyclase have been found to modulate (reduce) cellular radiosensitivity (Hinz 1982) the basal activity of this enzyme was measured systematically in four different cell lines under various conditions of growth. The results are given in Table 1. According to Dertinger and Hülser (1981) L-cells (mouse) are uncoupled, V79-cells (Chinese hamster) show weak coupling, whereas 3T3-cells (mouse) and B14 FAF28-cells (Chinese hamster) are strongly coupled. Striking differences in the pattern of cyclase activity are obvious between coupled and uncoupled cell lines. Whereas there is a gradual increase in activity from single cells to spheroids in the uncoupled L-cells, the coupled cell lines exhibit maximum activity in the single cells and minimum activity in the spheroids. Moreover, the results of the coupled cell lines (3T3 and B14) suggest that cyclase activity is reduced stepwise with increasing strength of cell contact (single cells → monolayer → spheroids) with only minor influence of the proliferative status. V79 shows an intermediate response between coupled and uncoupled cell lines: Following a drop in cyclase activity from single cells to exponentially growing monolayers the figure for confluent monolayers approaches that of the single cells.

Preliminary results indicate that the cellular cyclic AMP content follows the same pattern as the cyclase activity in Table 1.

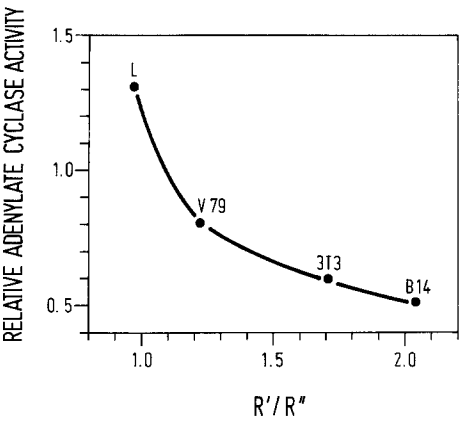

Fig. 2. Relative adenylate cyclase activity for different cell lines versus electrical coupling. Abscissa: Ratio (single cells/monolayer) of electrical input resistances; values taken from Dertinger and Hülser (1981). Ordinate: Enzymatic activities (Table 1) of monolayers (average of exponential and plateau cultures) relative to single cells

The correlation between cyclase activity and coupling suggested by Table 1 can be made more obvious, when cyclase activity is plotted against a quantity related to the degree of coupling. Figure 2 shows a graph of relative (monolayer/single cells) adenylate cyclase activity (Table 1) versus strength of coupling expressed as ratio of electrical input resistances (single cells/monolayer). A good correlation is obtained: The higher the coupling the lower the cyclase activity in monolayers relative to single cells. However, Fig. 2 does not explain whether the down-regulation of adenylate cyclase in monolayers is due to increased coupling (gap-junctional conductivity) or whether cyclase activity regulates the gap junctions. Alternatively, both could be controlled by other factors.

Since the low cyclase activity in the coupled spheroids cannot be explained in terms of proliferative effects we investigated whether functional changes of the gap-junctions (i.e., electrical conductivity) could eventually be associated with the sharp drop in enzyme activity. For this reason the distribution of the cellular electrical input resistances was measured in spheroids (outer cells) of B14 FAF28 cells. The mean value of the distribution is given in brackets in Table 1 together with the corresponding figures for monolayers and single cells. Evidenctly, the input resistance of spheroid cells is higher than that of monolayers suggesting weaker coupling in spheriods. This result is surprising since it seems to be incompatible with Fig. 2 and with the common imagination that coupling in a three-dimensional cell matrix should be higher (at least not lower) than in a monolayer. Thus a more rigorous investigation was initiated to test whether this decrease of coupling in spheroids could be related to the size (age) of the spheroids. Figure 3 shows the results of this study indicating an increase of input resistance with increasing spheroid volume. Clearly, small spheroids exhibit strong coupling (comparable to monolayers) in agreement with expectation but coupling decreases in a later stage of spheroid development which was also confirmed by three-electrode measurements. It should be mentioned in this context that the spheroid radioresistance in B14 cells is only observed beyond a critical volume of approximately 4×10^6 μm^3 (Hinz 1982), this means in a region where decoupling is already in progress (Fig. 3).

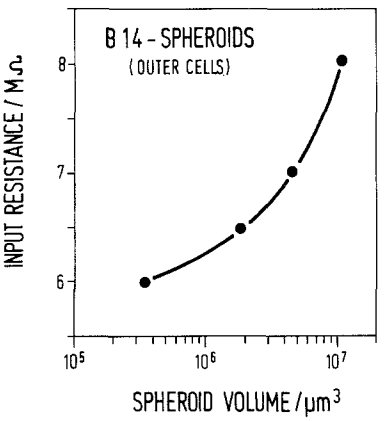

Fig. 3. Input resistance of outer cells in B14 FAF28 spheroids as function of spheroid volume. Measurements were made at 24-h intervals. The points represent mean values of five independent cultures with 200–300 cells measured per point

Conclusions

These results indicate that coupled cells cultured in vitro as multicellular spheroids may experience certain physiological alterations which are not observed in conventional monolayer culture. For example, electrical decoupling occurs after some time of "maturation" and the activity of adenylate cyclase falls to a level much lower than under any other culture condition. One consequence of this "new" physiological status of the spheroid cells is clearly the observed increase in radioresistance. But it may be expected that additional unidentified functional changes of the cells are associated with the spheroid culture system. As yet, the role of the tight three-dimensional contact of the cells within the spheroids in the expression of these phenomena is not understood.

References

Dertinger H, Hülser D (1981) Increased radioresistance of cells in cultured multicell spheroids. I. Dependence on cellular interaction. Radiat Environ Biophys 19: 101–107

Durand RE, Sutherland RM (1973) Growth and radiation survival characteristics of V79-171b Chinese hamster cells: A possible influence of intercellular contact. Radiat Res 56: 513–527

Guichard M, Dertinger H, Malaise EP (1982) Radiosensitivity of four human tumor xenografts. Influence of hypoxia and cell-cell contact. Radiat Res, in press

Hinz G (1982) Erhöhte Strahlenresistenz von Sphäroiden aus ionisch gekoppelten Zellen. Zytogenetische und biochemische Untersuchungen. Thesis, University of Heidelberg

Hülser DF, Webb DJ (1973) Relation between ionic coupling and morphology of established cells in culture. Exp Cell Res 80: 210–222

Jacobs KH, Saur W, Schultz G (1976) Reduction of adenylate cyclase activity in lysates of human platelets by the α-adrenergic component of epinephrine. J Cyclic Nucleotide Res 2: 381–392

Accepted October 8, 1982