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Tumor Cell Invasion and Gap Junctional Communication

I. Normal and Malignant Cells Confronted in Monolayer Cultures[1]

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Abstract. Mammary tumor cells of the rat (BICR/M1R$_k$) and mouse (EMT6/Ro) as well as rat glioma cells (C6) are electrically coupled and show intercellular dye spreading. Monolayer cultures of synchronously beating chicken heart cells were also electrically coupled, dye spreading, however, was significantly restricted to only one or two adjacent cells. In all coupled cells, gap junctions were found in both freeze-fracture replicas and ultrathin sections. Heterologous gap junctional coupling between these tumor cells and heart cells was regularly established. The human cervix carcinoma line HeLa and the mouse L sarcoma line were electrically not coupled and did not reveal gap junctions, consequently they showed no coupling to heart cells.

Introduction

Invasive tumor cells progressively occupy other tissues, causing degenerative alterations [19]. Despite the important role tumor cell invasion plays for malignancy, little is known about its mechanism. According to our present knowledge, a combined action of tumor cell proliferation, active locomotion and release of lytic enzymes results in a progressive degeneration of the normal host tissue [18, for review]. None of these three basic mechanisms, however, is per se considered specific for malignant

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cells. Tumor cell invasion into the surrounding normal tissue is an early step in the process of metastasis which, in most cases, is responsible for lethality caused by malignant tumors. A common prerequisite for invasive tumor cells is the ability to survive in the metabolic environment of the host tissue. Clinical data from human autopsy studies showed that tumors have patterns of metastatic colonisation depending on their original organ and histotype [30, for review]. Specific interactions between tumor cells and the normal host tissue may require a diffusion of soluble substances, promoting and increasing tumor cell attachment and survival [11].

Other mechanisms such as direct cell-to-cell contact are likely to be involved. In most vertebrate tissues, intercellular communication and cooperation are mediated by specialized membrane contacts between adjacent cells, so-called gap junctions. Specific proteinaceous channels bridge the gap between the adjacent cells and connect their cytoplasms. These channels allow a direct transfer of ions and small molecules up to a molecular weight of about 900 daltons [5], thus providing a path not only for intercellular electrical communication but also for metabolic cooperation [8, 29]. In living organisms, intercellular communication via gap junctions is involved in the synchronization of contracting heart muscle cells, in the regulation of growth, and in processes of differentiation and development [22, for review].

Gap junctions, however, do not only occur in normal tissues, but have also been described for a variety of tumor cells [12, 27]. Gap junctional communication has also been detected in mixed cultures of vertebrate cells derived from different tissues and different organisms [4, 9, 21]. Interestingly, coupled cells might be less sensitive to changes of the cell microenvironment or stress factors than cells without gap junctions as has been shown for irradiated multicellular tumor spheroids [2]. Loewenstein and Kanno [17] correlated the absence of gap junctional communication to tumorigenicity. This hypothesis has been modified and states now that 'the junctional membrane channels are instrumental in cell-to-cell transmission of molecules necessary for the control of growth' [15]. When tumor cells are coupled to normal cells, their proliferative capacity may be reduced [15, 32] or – as our results indicate – they may have an advantage for invading normal tissue.

In this paper, we report investigations with monolayer cultures of five different tumor cell lines. Their homologous gap junctional coupling capacity and their heterologous junctional communication with embryonic chick heart cells were measured. These data are fundamental for the inter-

pretation of the histological results described in the following paper where an in vitro invasion assay [20] was used to investigate whether the invasive behaviour of these tumor cell lines is correlated with their gap junctional coupling capacity.

Materials and Methods

Permanently Growing Cells

Monolayer cultures of five different tumor cell lines were used in this study. Fibroblastoid BICR/M1R$_k$ cells were derived from the BICR/M1R transplantable mammary tumor of the Marshall rat [24]. The fibroblastoid EMT6/Ro cells were selected from a mammary tumor cell line of a Balb/c mouse [25]. Fibroblastoid C6 cells were derived from N-nitrosomethylurea-induced glial tumors of the rat [1]. HeLa cells were established from a human cervix carcinoma [7, 26]. Fibroblastoid L cells originated from a 20-methylcholanthrene-treated primary strain of C3H-mouse-fibroblasts [3].

Primary Embryonic Chick Heart Cells

Single cell suspensions of 9-day-old embryonic chick hearts were prepared as described by Freshney [6]. Briefly, chick hearts were explanted from 9-day-old embryos, placed overnight in 1 ml ice-cold trypsin (0.25% trypsin in phosphate-buffered saline (PBS) without calcium and magnesium). After trypsin removal, the organ was incubated in the residual trypsin at 37 °C for 10–15 min and dispersed by gentle pipetting in 2 ml culture medium. The cell suspension was then seeded in 3 to 4 60-mm diameter Petri dishes (Falcon, 3002 F, Becton Dickinson, Mountainview, Calif.) with 5 ml culture medium.

Culture Conditions

All cell lines were grown at pH 7.4 and 37 °C in a humidified incubator with 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (Biochrom KG, Berlin, FRG) supplemented with 3.7 g/l $NaHCO_3$, 100 mg/l streptomycine sulfate, 150 mg/l penicillin G and 5% calf serum. Cells were passaged in tissue culture flasks (Nunc, Roskilde, Denmark) by treatment with 0.25% trypsin in PBS without calcium and magnesium.

For electrophysiological experiments the cells were cultured in Petri dishes (Falcon 3002 F). The bicarbonate/CO_2-buffered culture medium was replaced for Hepes-buffered medium (25 m*M*) only for embryonic heart cells during measurements.

Confrontation cultures of embryonic chick heart cells with permanently growing mammalian tumor cells were obtained by seeding tumor cells to subconfluent heart monolayer cultures. For better identification, tumor cells had been stained with vital red (0.8 mg/ml culture medium; Chroma, Köngen, FRG) for 2 to 3 days prior to trypsinization and subsequent confrontation.

Electrophysiology

Measurements of membrane potentials and intracellular applications of electrical current were performed under light microscopical observation (Leitz Epivert or Zeiss Standard RA, equipped with phase contrast and epifluorescence illumination). Glass

microelectrodes were pulled from capillary glass (outer diameter 1 mm, with inner filament; Hilgenberg Glas, Malsfeld, FRG) with a pipette puller (DK Instruments, Tujunga, Calif.) and back-filled with 3 *M* KCl; their tip resistances varied between 20 and 30 MΩ. For measurements of membrane potentials, the two recording microelectrodes were connected through an Ag/AgCl-electrode to the input of a capacity-compensated electrometer (750, WP Instruments, New Haven, Conn.). For current injection, a microelectrode was connected to an electrometer (M 701, WP Instruments) and rectangular hyperpolarizing current pulses of 5 nA and up to 50 ms duration, supplied by a stimulator (TWG 501, Feedback Instruments, Crowborough, UK) were injected into a cell. The resulting hyperpolarizing pulses were registered in the same and, in case of coupling, in adjacent cells [16]. Membrane potentials and current amplitudes were displayed on a digital storage oscilloscope (Nicolet 3091, Madison, Wisc.) and either photographed from the screen or recorded with an X-Y-recorder (F-42 CP, Rikadenki Kogyo Co., Tokyo, Japan). In addition to measurements of ionic coupling, we investigated whether larger molecules would pass through gap junctions. We therefore applied microiontophoresis, using glass microelectrodes back-filled with a 4% solution of the fluorescent dye Lucifer yellow CH (M_r 457, Sigma, St. Louis, Mo.) in 1 *M* LiCl. Cells were injected for 30 s with a negative current step of up to 20 nA, supplied by the iontophoresis unit of a microelectrode amplifier (L/M-1, List electronic, Darmstadt, FRG). The progress of microinjection was monitored on a Zeiss Standard microscope under epifluorescence illumination (exciter filter BP 450-490, dichroic mirror 510, barrier filter LP 520) and photographed after 2 min on an Ilford HP 5 film. To reduce background fluorescence, the culture medium was exchanged for PBS. In all electrophysiological experiments micromanipulators with electrical drives (Gebr. Märzhauser, Wetzlar, FRG) allowed a controlled positioning of microelectrodes.

Electron Microscopy

Monolayer cell cultures were washed twice in PBS and fixed in 2.5% glutardialdehyde (Merck, Darmstadt, FRG) for 30–60 min at room temperature. For thin sections the cells were fixed in 1% osmium tetroxide (Merck) and embedded in epoxy resin Glycidether 100 (formerly Epon 812, C. Roth, Karlsruhe, FRG). Regions of interest were selected, polymerized as small cubes onto resin capsules, thin sectioned and poststained with uranyl acetate and lead citrate. For freeze fracture the cells were treated with increasing concentrations of glycerol and rapidly frozen as cell pellets in liquid Freon 22, freeze fractured and replicated under high vacuum in a BAF 301 instrument (Balzers AG, Liechtenstein). Thin sections and replicas were examined with a Zeiss EM 10 A electron microscope at 60 kV.

Results

Intercellular Coupling

Ionic coupling was detected in BICR/M1R_k, EMT6/Ro and C6 cultures (fig. 1a, d, g). However, iontophoretical injection of the fluorescent dye Lucifer yellow into these monolayer cultures revealed differences in the degree of dye coupling.

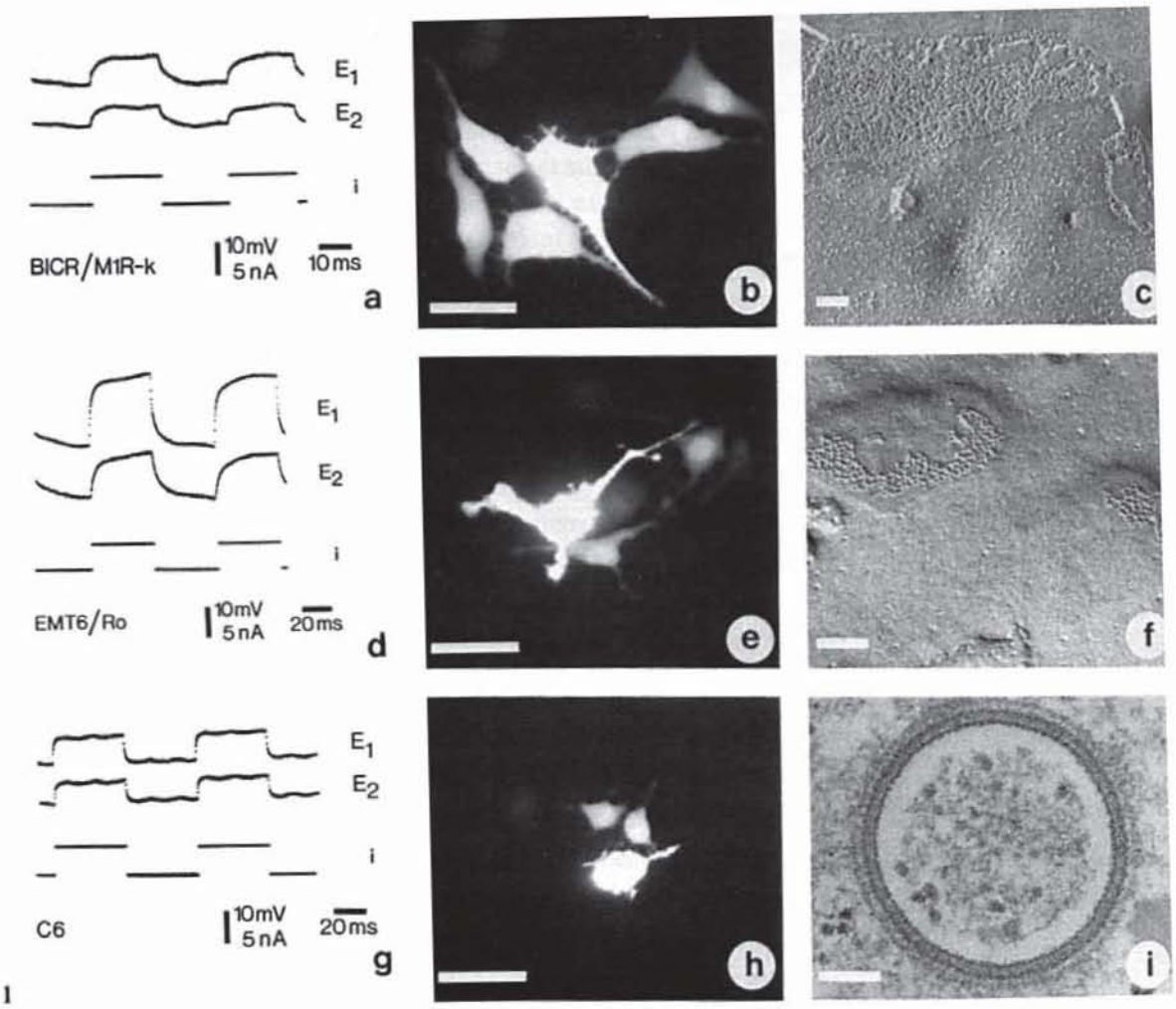
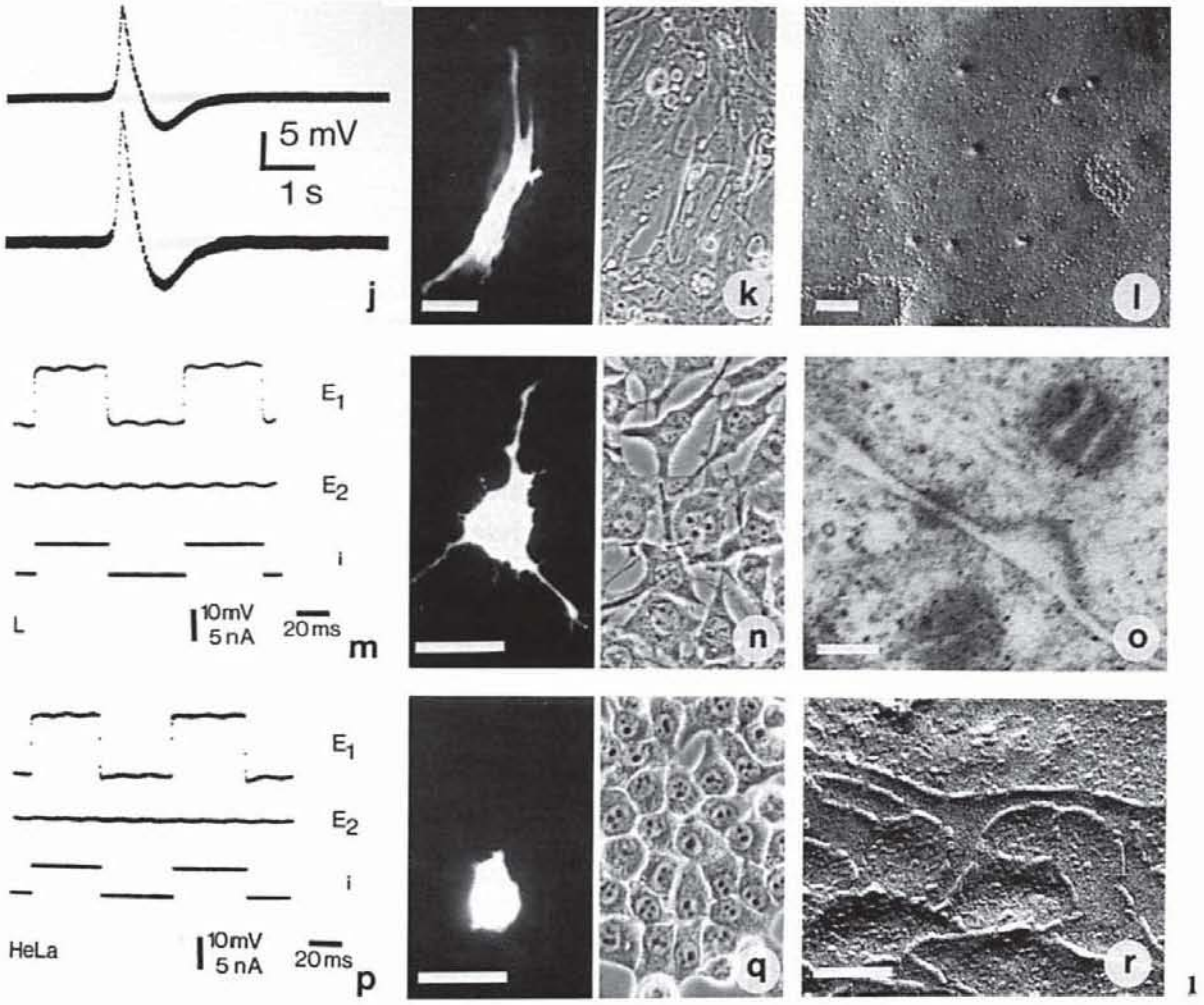


Fig. 1. Comparative electrophysiological and ultrastructural investigations on monolayer cultures of BICR/M1R$_k$ cells (**a–c**), EMT6/Ro cells (**d–f**), C6 cells (**g–i**), embryonic chick heart cells (**j–l**), L cells (**m–o**), and HeLa cells (**p–r**).

Left column: measurements of ionic coupling. Oscilloscope photographs (**a, d, g, m, p**) depict rectangular hyperpolarizing current pulses (**i**, bottom trace) of a stimulating electrode impaled in one cell of a monolayer culture and the responding membrane potential of the injected (top trace, E_1) and an adjacent cell (middle trace, E_2).

After dye injection into a BICR/M1R$_k$ cell dye spreading into all contacting cells within a large area of the monolayer culture could be observed (fig. 1b). In contrast, dye injection into an EMT6/Ro cell resulted in a different pattern of dye spreading: the fluorescent dye could only be detected in some of the attached cells (fig. 1e). In C6 glioma cells, dye coupling was even more restricted, in most cases fluorescence was apparent in only one or two directly connected cells (fig. 1h).



Middle column: dye coupling measurements in epifluorescence illumination; **k**, **n** and **q** with the same cells in phase contrast microscopy. Bar: 50 μm.

Right column: electron micrographs show freeze-fracture replicas of gap (**c**, **f**, **l**) and tight junctions (**r**) as well as thin sections of an annular gap junction (**i**) and a desmosome (**o**). Bar: 0.1 μm.

Measurements of ionic coupling between embryonic chick heart cells in monolayer culture were performed with only two voltage recording microelectrodes. The heart cell action potentials were used as an intrinsic voltage signal, transmitted through gap junctional channels into coupled neighbor cells: from synchronously beating heart cells synchronous action potentials were recorded (fig. 1j). Injection of the fluorescent dye Lucifer yellow, however, revealed only a minor degree of dye

coupling: dye transfer was restricted to one or two contacting cells (fig. 1k).

In contrast, with L and HeLa cells neither ionic coupling (fig. 1m, p) nor dye coupling could be registered: the injected cells retained the dye, no matter how many cells were in direct contact with the injected cell (fig. 1n, q).

Electron Microscopical Investigations

All cell cultures were processed for ultrastructural investigation of plasma membrane contacts between adjacent cells. With BICR/M1R_k cells, many gap junctions were found in freeze-fracture replicas (fig. 1c). A less frequent appearance of gap junctions was observed with EMT6/Ro cells (fig. 1f). Very rarely, gap junctions were detected in ultrathin sections of C6 cells. In most cases, these gap junctions were of the annular type (fig. 1i). In freeze-fracture replicas of embryonic chick heart cells, gap junctions appeared as irregularly shaped plaques of small size (fig. 1l). In thin sections and freeze-fracture replicas of the fibroblastoid cell line L and the epithelioid cell line HeLa, which did not exhibit ionic or dye coupling, gap junctions were not detected.

Whereas L cells only exhibited desmosome-like structures as specialized cell junctions (fig. 1o) HeLa cells revealed numerous tight junctions in freeze-fracture replicas (fig. 1r).

Measurements of Heterologous Ionic Coupling in Mixed Monolayer Cultures

Monolayer confrontation cultures of embryonic chick heart cells with each of the five investigated mammalian cell lines were tested for heterologous gap junctional communication. In these mixed cultures, the vital red-labeled mammalian cells could be unequivocally distinguished from the heart cells by dye granules accumulated around the nuclei of the labeled cells. As the amount of dye decreased with each cell division, all experiments were carried out within 20–30 h after addition of labeled cells to the precultured heart monolayers.

In confrontation cultures of heart cells with the coupled cell lines BICR/M1R_k, EMT6/Ro and C6, the experiments were performed by inserting a potential recording microelectrode into a labeled cell adjacent to a heart muscle cell. The spontaneous heart action potentials were used as endogenous signals which could be detected in neighboring tumor cells when they were coupled by gap junctions to the heart cells. All cell lines

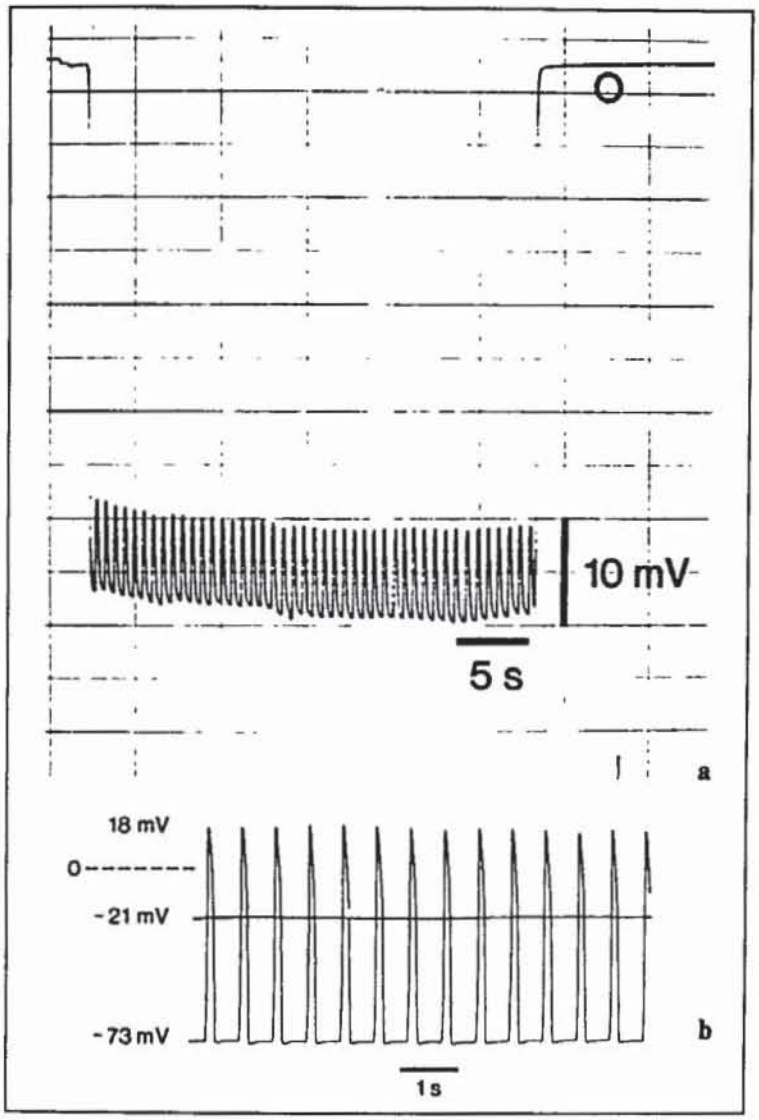


Fig. 2. Measurements of heterologous ionic coupling in monolayer confrontation cultures of embryonic chick heart cells with tumor cells. **a** Action potentials of a heart cell detected in an adjacent BICR/M1R_k cell. **b** The membrane potential of a HeLa cell (−21 mV) did not exhibit any superposition with the simultaneously measured action potentials of an adjacent heart cell.

which were ionically coupled in homologous monolayer cultures (fig. 1a, d, g), also coupled to embryonic heart cells, as shown in figure 2a for a BICR/M1R_k cell. Chick heart action potentials detected in coupled mammalian cells reached values between 2 and 8 mV, depending on cell density and ratio of tumor to heart cells, the frequencies varied with the temperature of the culture medium.

In confrontation cultures of embryonic chick heart cells with the non-coupled cell lines L and HeLa, both the membrane potentials of the tumor cells and the action of the adjacent heart cell were recorded. In these cultures heterologous coupling could never be recorded, an example is shown in figure 2b, where a HeLa cell exhibited a constant membrane potential of −21 mV, although the adjacent heart cells generated action potentials with amplitudes of about 90 mV.

Discussion

Ionic coupling, indicating intercellular spreading of ions such as Na^+, K^+ and Cl^-, could not only be detected in a mammary tumor of the rat (BICR/M1R_k) – as already described [12] – but also in the EMT6/Ro mouse mammary tumor and in C6 rat glioma. Our results are in agreement with a number of studies demonstrating that tumor cells of various origin are able to communicate via gap junctions [9, 12, 27]. Microinjection of the fluorescent dye Lucifer yellow CH, however, revealed differences in the amount of dye spreading into neighboring cells. Numerous fluorescent cells in monolayers of BICR/M1R_k-cells correlated well with the presence of numerous gap junctions in thin sections and freeze-fracture replicas.

An irregular pattern of dye transfer could be observed in EMT6/Ro monolayer cultures, frequently exhibiting a selective spreading into some of the neighboring cells. This restriction of dye transfer coincided with a less frequent appearance of gap junctions in electron microscopic preparations, suggesting that the number of gap junctions could be a factor for the regulation of intercellular communication. This view was supported by the detection of an extremely small number of gap junctions in thin sections and the unsuccessful search for these structures in freeze-fracture replicas of C6 cells. These cells exhibited the lowest degree of dye spreading, in most cases fluorescence could be detected in only one or two direct neighbor cells. Annular gap junctions found in these cells revealed an electron dense filamentous coat around the gap junctional membrane (fig. 1i). In electron microscopical investigations with a number of cells of different origin, Larsen et al. [14] demonstrated that this coat is composed of actin filaments being involved in the internalization of gap junction structures in vesicular form. A low proportion of communicating gap junctions – compared with a much higher number of internalized gap junctions – could result in a reduced number of intercellular channels which, however,

might still be sufficient to allow ionic coupling, but reduce dye spreading to a subthreshold extent. Another possible explanation for differences in the degree of dye coupling could be a gradual closing of gap junctional channels, as has been described for BICR/M1R$_k$ multicell spheroids [13]. In these cell aggregates, ionic coupling could be registered between cells which allowed no longer the transfer of dye, demonstrating that small current-carrying ions could pass through the channels whereas the much larger Lucifer yellow CH molecules (M_r 457) were retained. In this study we obtained similar results with embryonic chick heart cells, which regularly exhibited ionic coupling in monolayer culture, whereas dye spreading was limited to one or two adjacent cells. A lack of dye spreading has been reported for the smaller sodium fluorescein dye molecules (M_r 332) injected into ionically coupled atrioventricular cells of mammalian heart [23]. Williams and De Haan [31] calculated from electrophysiological data that only 5 gap junctional channels would allow synchronization of beating heart cells. In dye spreading experiments, however, the amount of fluorescent dye molecules transferred through only a few channels might be too small to be detected by fluorescence microscopy.

Neither dye spreading nor ionic coupling could be detected for L and HeLa cells, which correlated with the lack of gap junctions in electron microscopical preparations. HeLa cells were linked by numerous tight junctions (fig. 1r), which are necessary to maintain a selective permeability barrier [28] in epithelia.

Since measurements of ionic coupling revealed a higher sensitivity than the dye spreading assay, monolayer cultures of embryonic chick heart cells confronted with each of the 5 tumor cell lines were screened for heterologous junctional communication by measurements of ionic coupling. In confrontation cultures with the coupling-competent tumor cell lines BICR/M1R$_k$, C6, and EMT6/Ro action potentials of an adjacent heart cell could be recorded in the respective tumor cell. In our experiments the voltage deflections of heart action potentials detected in tumor cells correspond well with results of Goshima [9], who recorded excitation potentials from nonexcitable cells which were cocultured with myocardial cells and suggested an electrotonic transmission mechanism.

In homogeneous cultures of HeLa and L cells we could not detect gap junctional coupling, consequently, no heterologous coupling could be measured between embryonic chick heart cells and HeLa or L cells. These results confirm the finding of Goshima [9] who did not observe synchronization of independently beating mouse myocardial cells across bridging

L cells. This lack of heterologous communication corresponded with the absence of gap junctions between heart and L cells in thin sections [10]. Furthermore, the lack of depolarization potentials in noncoupled tumor cells is a strong argument against the possibility that a nonjunctional current might be responsible for the rhythmical depolarization in coupled tumor cells adjacent to a beating heart cell.

These results demonstrate that under our experimental conditions coupling-competent tumor cells – regardless of their tissue of origin – are able to form open gap junctional channels with embryonic chick heart cells. Some consequences of these heterologous gap junctions will be discussed in the following paper, where all five cell lines described in this paper were investigated for their invasive capacities in a well-established [20] three-dimensional in vitro invasion assay using embryonic chick heart cells as host tissue.

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References

1 Benda, P.; Lightbody, J.; Sato, G.; Levine, L.; Sweet, W.: Differentiated rat glial cell strain in tissue culture. Science *161:* 370–371 (1968).

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

3 Earle, W.R.; Schilling, E.L.; Stark, T.H.; Straus, N.P.; Brown, M.F.; Shelton, E.: Production of malignancy in vitro. IV. The mouse fibroblast cultures and changes seen in the living cells. J. natn. Cancer Inst. *4:* 165–212 (1943).

4 Epstein, M.L.; Gilula, N.B.: A study of communication specificity between cells in culture. J. Cell Biol. *75:* 769–787 (1977).

5 Flagg-Newton, J.; Simpson, J.; Loewenstein, W.R.: Permeability of the cell-to-cell-membrane channels in mammalian cell junctions. Science *205:* 404–407 (1979).

6 Freshney, R.I.: Disaggregation of the tissue and primary culture; in Freshney, Culture of animal cells. A manual of basic technique, pp. 99–118 (Alan R. Liss, New York 1984).

7 Gey, G.O.; Coffman, W.D.; Kubicek, M.T.: Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res. *12:* 264–265 (1952).

8 Gilula, N.B.; Reeves, O.R.; Steinbach, A.: Metabolic coupling, ionic coupling and cell contacts. Nature, Lond. *235:* 262–265 (1972).
9 Goshima, K.: Synchronized beating of and electrotonic transmission between myocardial cells mediated by heterotypic strain cells in monolayer culture. Exp. Cell Res. *58:* 420–426 (1969).
10 Goshima, K.: Formation of nexuses and electrotonic transmission between myocardial and FL cells in monolayer culture. Exp. Cell Res. *63:* 124–130 (1970).
11 Horak, E.; Darling, D.; Tarin, D.: Organ-specific effects on metastatic tumor growth studied in vitro; in Hellmamm, Eccles, Treatment of metastasis: problems and proposals, pp. 369–372 (Taylor & Francis, London 1985).
12 Hülser, D.F.; Webb, D.J.: Relation between ionic coupling and morphology of established cells in culture. Exp. Cell Res. *80:* 210–222 (1973).
13 Hülser, D.F.; Brümmer, F.: Closing and opening of gap junctions pores between two- and threedimensionally cultured tumor cells. Biophys. Struct. Mech. *9:* 83–88 (1982).
14 Larsen, W.J.; Tung, H.N.; Murray, S.A.; Swenson, C.A.: Evidence for the participation of actin microfilaments and bristle coats in the internalization of gap junction membrane. J. Cell Biol. *83:* 576–587 (1979).
15 Loewenstein, W.R.: Junctional intercellular communication and the control of growth. Biochim. biophys. Acta *560:* 1–65 (1979).
16 Loewenstein, W.R.; Kanno, Y.: Studies on an epithelial (gland) cell junction. I. Modifications of surface membrane permeability. J. Cell Biol. *22:* 565–586 (1964).
17 Loewenstein, W.R.; Kanno, Y.: Intercellular communication and tissue growth. I. Cancerous growth. J. Cell Biol. *33:* 225–234 (1967).
18 Mareel, M.; Kint, J.; Meyvisch, C.: Methods of study of the invasion of malignant C3H-mouse fibroblasts into embryonic chick heart in vitro. Virchows Arch. Abt. B Zellpath. *30:* 95–111 (1979).
19 Mareel, M.M.: Recent aspects of tumor invasiveness. Int. Rev. exp. Pathol. *22:* 65–129 (1980).
20 Mareel, M.M.: Invasion in vitro. Methods of analysis. Cancer Metastasis Rev. *2:* 201–218 (1983).
21 Michalke, W.; Loewenstein, W.R.: Communication between cells of different type. Nature, Lond. *232:* 121–122 (1971).
22 Pitts, J.D.; Finbow, M.E.: The gap junction. J. Cell Sci. *4:* suppl., pp 239–266 (1986).
23 Pollack, G.H.: Intercellular coupling in the atrioventricular node and other tissues of the rabbit heart. J. Physiol., Lond. *255:* 275–298 (1976).
24 Rajewsky, M.F.; Grüneisen, A.: Cell proliferation in transplanted rat tumors: influence of the host immune system. Eur. J. Immunol. *2:* 445–447 (1972).
25 Rockwell, S.C.; Kallman, R.F.; Fajardo, L.S.: Characteristics of a serially transplanted mouse mammary tumor and its tissue-culture-adapted derivative. J. natn. Cancer Inst. *49:* 735–749 (1972).
26 Scherer, W.F.; Syverton, J.T.; Gey, G.O.: Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix. J. exp. Med. *97:* 695–709 (1953).

27 Sheridan, J.D.: Low resistance junctions between cancer cells in various solid tumors. J. Cell Biol. *45:* 91–99 (1970).
28 Staehelin, L.A.; Hull, B.E.: Junctions between living cells. Scient. Am. *238:* 141–152 (1978).
29 Subak-Sharpe, J.H.; Bürk, R.R.; Pitts, J.D.: Metabolic cooperation between biochemically marked cells in tissue culture. J. Cell Sci. *4:* 353–367 (1969).
30 Tarin, D.: Clinical and experimental studies on the biology of metastasis. Biochim. biophys. Acta *780:* 227–235 (1985).
31 Williams, E.H.; De Haan, R.L.: Electrical coupling among heart cells in the absence of ultrastructurally defined gap junctions. J. Membrane Biol. *60:* 237–248 (1981).
32 Yamasaki, H.; Katoh, F.: Novel method for selective killing of transformed rodent cells through intercellular communication, with possible therapeutic applications. Cancer Res. *48:* 3203–3207 (1988).

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