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Perspectives and Commentaries

Mechanisms of Tumor Promotion: Possible Role of Inhibited Intercellular Communication*

JAMES E. TROSKO

Department of Pediatrics/Human Development, College of Human Medicine, Michigan State University, East Lansing, Michigan 48824, *U.S.A.*

(A COMMENT ON: Arenholt D., Philipsen H.P., Nikai H., Andersen L., Jepsen A. Chemically unrelated tumor promoters induce identical morphological changes in cultured rat oral epithelium. *Eur J Cancer Clin Oncol*, 1987, **23**, 19–29.)

IN THE recent paper by Arenholt *et al.* (*Eur J Cancer Clin Oncol* 1987, **23**, 19–29) *in vitro* observations related to the effects of several structurally unrelated tumor-promoting chemicals were interpreted as implicating the role of gap junctional intercellular communication in the *cellular* mechanism of tumor promotion. They observed that several tumor-promoting chemicals, but not the non-tumor promoters, caused many cytomorphological effects in primary rat tongue squamous epithelial cells. In this "Commentary", I use this series of observations, and those of many diverse fields, such as developmental biology, chemical carcinogenesis, and molecular oncology, to attempt an integration of several current concepts; namely the concepts of "initiation/promotion", "gap juctional intercellular communication" and "oncogenes".

In spite of obvious criticisms that the Arenholt *et al.* observations were on cells, grown *in vitro* (tumor promotion is an *in vivo* operational concept), and derived from rats (an animal not yet shown to be particularly sensitive to skin promotion by the agents used in this study, although rat liver is an excellent species/organ system for promotion studies), I feel their results are consistent with other observations showing that many tumor promoting chemicals do inhibit intercellular communication both *in vitro* and *in vivo*.

The multi-step nature of carcinogenesis has been, operationally, conceptualized to consist of an "initiation" phase, in which a normal cell has its genome irreversibly altered to form a single pre-malignant or "initiated" cell by a single low dose of a "carcinogen", and a promotion phase, in which the single initiated cell is clonally multiplied by a variety of conditions, thereby helping to facilitate the conversion of the pre-malignant phenotype to a malignant one [1]. Although the molecular mechanisms for either of these processes are still unknown, there is strong evidence implicating *mutagenesis* as the basis for initiation [2]. To explain the clonal nature of cancer, and to be consistent with the operational definition of promotion, the single initiation cell must be increased. Therefore, among other things which might happen during the promotion phase, *mitogenesis* must occur to allow the selective increase of the initiated cell.

Again, while the molecular mechanism for mitogenesis is not known, the control of cell division and differentiation of potential proliferable cells (i.e. stem cells) seems to be linked to the phenomenon of "contact inhibition". Normal cells seem to "contact-inhibit" while cancer cells do not [3]. Here is where a possible connection to the concept of gap junctional intercellular communication is possible. The membrane-bound protein channel, gap junction, which regulates the intercellular transfer of ions and small molecular weight molecules, has been postulated to play a significant role in the regulation of proliferation and differentiation [4].

Interestingly, many cells derived from tumors have some dysfunction in their ability to perform gap junctional intercellular communication *in vitro* [5]. A decrease of gap junctions in tumor tissue *in vivo* has been noted [6]. In addition, it has been shown that many tumor promoting chemicals inhibit gap junctional communication *in vitro* [7,

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8] and that altered gap junctional communication is found during *in vitro* transformation [9]. Furthermore, it also has been demonstrated that the tumor-promoting phorbol ester reduces the frequency of gap junctions in mouse skin [10]. Partial hepatectomy, a tumor-promoting stimulus triggering regenerative hyperplasia in the rat, has also been shown to reduce gap junctions during the regenerative growth phase [11].

With the recent investigations in the area of oncogenes and carcinogenesis, another connection to gap junctions can be made. Cancer has been described as a "disease of differentiation" [12]. In the last few years the expression of various oncogenes has been correlated with a cell's commitment to either proliferate or differentiate [13]. Several oncogene products, such as from the src and ras, now have been shown to be associated with a decrease in gap junctional communication [14–16]. One could speculate that other oncogene products associated with growth factor-like activity (e.g. sis) or growth factor receptors (e.g. erb-B) might also affect gap junction communication in order for these gene products to block contact-inhibition.

On the molecular/biochemical level, a major hypothesis has been offered to explain the mechanism of tumor promotion, namely the activation of protein kinase C (PK-C) [17]. With the observation that the phorbol ester-like tumor promoters have the ability to activate PK-C, the demonstration that the physiological activators for PK-C, diacylglycerol, could inhibit gap junctional communication [18] adds further evidence to the hypothesis that PK-C and gap junctional communication are linked to tumor promotion.

Together with the fact that gap junction communication occurs with the appearance of the ability of multi-cellular organisms to form differentiated functions [19], the fact that gap junctional communication can be modulated by developmental factors, endogenous hormones, and environmental chemicals suggests that it plays an important adaptive function [7].

Conditions which modulate intracellular levels of Ca^{2+}, pH and cyclic AMP have been correlated with modulation of gap junction function [20], providing an explanation for a wide variety of structurally unrelated chemicals which influence tumor promotion. In other words, these various chemicals, by ultimately increasing intracellular Ca^{2+} or H^+ ion concentrations by different mechanism, can inhibit intercellular communication. On the other hand, those chemicals which increase C-AMP levels appear to be able to increase intercellular communication [21].

One might ask how can tumor promoting conditions and chemicals, by inhibiting gap junctional communication, trigger cells to proliferate or differentiate, as has been seen *in vitro* and *in vivo*. As has been postulated, homeostatic equilibrium of critical ions (Ca^{2+}, H^+) and regulatory molecules (e.g. C-AMP) is maintained by gap junctional communication in a tissue of contact-inhibited cells [7, 19]. These potentially proliferable cells, being quiescent, are in the G_0 state of the cell cycle. Mitogenic agents (growth factors, wound-healing factors, tumor promoters, etc.) must alter the cell, via the transmembrane cascade of biochemical signals, to (a) become permeable to the small molecular weight building blocks for macro-molecular synthesis; (b) facilitate proper ionic balance to favor the physiological transition from the G_0 to G_1 state; and (c) prevent high energy-containing molecules synthesized for macromolecular products from leaving the cell via gap junctions by eliminating gap-junctional transfer [7].

This coordinated series of actions allows the initiated cell (as well as normal cells) to escape contact inhibition and to proliferate. By proliferating, an initiated stem cell, which is somehow blocked in its ability to terminally differentiate [22], would not only increase in numbers, but by the process of proliferating increase its chances of accumulating additional phenotypic changes due to genotypic alterations which only occur during replication.

If, in fact, this hypothesis is correct, there are significant practical implications for cancer therapy. If tumor promotion involves the inhibition of gap junctional communication, and if certain oncogene products are involved in the inhibition of gap junction function, and if malignant cells are unable to perform gap junctional communication, conceptually it should be conceivable to induce gap junctional communication in these non-communicating malignant cells, forcing them to contact inhibit and possible to terminally differentiate. Therefore, a new theoretical strategy for chemotherapy should include agents which can *increase* gap junctional communication in cancer cells. This clearly implies new concepts of "anti-promoters" and "anti-oncogenes".

Finally, more work must be undertaken to study the basic mechanisms of gap junctional communication in order to understand species/organ differences in response to chemicals and to modulate gap junctional communication for purposes of cancer chemotherapy.

REFERENCES

1. Argyris T. Regeneration and the mechanism of epidermal tumor promotion. *CRC Crit Rev Toxicol* 1985, **14**, 211–258.
2. Quintanilla M, Brown K, Ramsden M, Balmain A. Carcinogen-specific mutation and amplification of Ha-ras during mouse skin carcinogenesis. *Nature* 1986, **322**, 78–79.
3. Borek C, Sachs L. The difference in contact inhibition of cell replication betwen normal cells and cells transformed by different carcinogens. *Proc Natl Acad Sci USA* 1966, **56**, 1705–1711.
4. Loewenstein WR. Junctional intercellular communication and the control of growth. *Biochim Biophys Acta Cancer Rev* 1979, **560**, 1–65.
5. Kanno Y. Modulation of cell communication and carcinogenesis. *Japan J Physiol* 1985, **35**, 693–707.
6. Swift JG, Mukherjee TM, Rowland R. Intercellular junctions in hepatocellular carcinoma.*J Submicrosc Cytol* 1983, **15**, 799–810.
7. Trosko JE, Chang CC. Adaptive and non-adaptive consequences of chemical inhibition of intercellular communication. *Pharm Rev* 1984, **B6**, 137–144.
8. Trosko JE, Chang CC, Medcalf A. Mechanism of tumor promotion: potential role of intercellular communication. *Cancer Invest* 1983, 511–526.
9. Enomoto T, Yamasaki H. Lack of intercellular communication between chemically transformed and surrounding nontransformed Balb/c 3T3 cells. *Cancer Res* 1984, **44**, 5200–5203.
10. Kalimi GH, Sirsat SM. The relevance of gap junctions to stage 1 tumor promotion in mouse epidermis. *Carcinogensis* 1984, **5**, 1671–1677.
11. Meyer DJ, Yancey SB, Revel JP. Intercellular communication in normal and regenerating rat liver. A quantitative analysis. *J Cell Biol* 1981, **91**, 505–523.
12. Pierce GB. Neoplasms, differentiation and mutations. *Am J Pathol* 1974, **77**, 103–118.
13. Weinberg RA. The action of oncogenes in the cytoplasm and nucleus. *Science* 1985, **230**, 770–776.
14. Atkinson M, Sheridan J. Decreased junctional permeability in cells transformed by 3 different viral oncogenes: a quantitive video analysis. *J Cell Biol* 1984, **99**, 401a.
15. Azarnia R, Loewenstein WR. Intercellular communication and the control of growth: alteration of junctional permeability by the src gene. A study with temperature-sensitive mutant Rous sarcoma virus. *J Membr Biol* 1984, **82**, 191–205.
16. Chang CC, Trosko JE, Jung HJ, Bomick D, Matsumura F. Potential role of the src gene product in inhibition of gap junctional communication in NIH 3T3 cells. *Proc Natl Acad Sci USA* 1985, **82**, 5360–5364.
17. Nishizuka Y. Studies and perspectives of protein kinase C. *Science* 1986, **233**, 305–312.
18. Gainer H St C, Murray AW. Diacylglycerol inhibits gap junctional communication in cultured epidermal cells: evidence for a role of protein kinase C. *Biochem Biophys Res Commun* 1985, **126**, 1109–1113.
19. Finbow ME, Yancey SB. The roles of intercellular junctions. In: Clemens MJ ed. *Biochemistry of Cellular Regulation.* Boca Raton, Florida, CRC Press, 1981, Vol. 4, 215–249.
20. Spray DC, Bennett MVL. Physiology and pharmacology of gap junctions. *A Rev Physiol* 1985, **47**, 281–303.
21. Saez JC, Spray DC, Nairn AC, Herzberg E, Greengard P, Bennett MVL. C-AMP increases junctional conductance and stimulates phosphorylation of the 27-KDa principal gap junction polypeptide. *Proc Natl Acad Sci USA* 1986, **83**, 2473–2477.
22. Yuspa SH, Morgan DL. Mouse skin cells resistant to terminal differentiation associated with initiation of carcinogenesis. *Nature* 1981, **293**, 72–74.