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19. ABSTRACT (Continue on reverse if necessary and identify by block number)
Progress during the first 12 months of this grant has progressed on all six specific aims, namely to study the basic mechanisms by which toxic chemicals block cell-cell communication; role of oncogenes in down-regulating gap junctional intercellular communication (GJIC); how protein kinase C enzyme, after activated by chemicals, down regulates GJIC; validate known toxic chemicals' ability to block GJIC in new human cell lines; isolate gap junction antibodies to characterize and study how gap junctions are regulated; and to isolate and characterize gap junction mutants. Several experimental, theoretical and review articles have been submitted. Presented research at recent international meetings and several national meetings.

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Annual Technical Report

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The Role of Chemical Inhibition of Gap-Junctional
Intercellular Communication in Toxicology

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I. SUMMARY OF PROGRESS TO DATE

The first three years of this grant were to validate the hypothesis that modulation of gap junctional intercellular communication by various chemicals could explain, in part, their mechanism of toxicity. Included in that effort was the need (a) to develop various in vitro cell model systems; (b) to develop new techniques to measure gap junction function; and (c) to test if various known toxicants (i.e., neurotoxicants, tumor promoters, reproductive toxicants) blocked gap junction function.

During the first year, we have continued to expand on the previous progress of the second phase of this grant (year 4) (with the in vitro cell system; "FRAP analysis, scrape-loading/dye transfer techniques) to study the molecular/ biochemical mechanisms by which chemicals block cell-cell communication and to develop new in vitro/in vivo models to study the biological consequences of stably-inhibited gap junctions using genetic mutants for gap junction function and oncogene-down regulated gap junctions.

Hopefully, the significance of this line of research will help understand how toxicant chemicals work, as well as help to develop in vitro alternatives to animal toxicity tests.

Specific Aims

Aim 1. We have now characterized a human kidney cell line for its ability to perform gap junction function. The rationale is to determine if there are species/organ specific chemical inhibitors of gap junction function. Paper has been submitted and accepted for publication in In Vitro Toxicology, (see enclosed preprint).

Aim 2.

1. In order to see how oncogenes might affect intercellular communication, we have transfected/infected with retroviruses, several cell lines. Several new in vitro/in vivo model systems are being developed.

A. Rat liver "oval" or epithelial cell line, infected with ras, metallothionein promoter, ras, raf, and neu oncogenes have been isolated or partially characterized. All of these have reduced gap junction function and form tumors in the liver, in the syngeneic rats within weeks, whereas the normal communication-proficient cells do not.

In collaboration with Dr. Mike Liebermann, University of Baylor School of Medicine, we have studied a metal promoter H-Ras transfected into a rat liver epithelial cell. The cell is normal,

non-tumorigenic and gap junction proficient when not exposed to zinc (a metal that turns on the ras oncogene). When exposed to zinc, cell communication is reduced, cells become transformed and tumorigenic. We are in the final phase of characterizing these cells (see enclosed data showing how expression of the Harvey ras oncogene is correlated with down regulation of cell-cell communication. Paper will be prepared soon.

We have also made a major advance in understanding the role of the Harvey-ras oncogene in blocking intercellular communication in both rat liver and Chinese hamster cells. In both cases, the activation of the H-ras oncogene in communicating, non-tumorigenic cells caused them to have reduced communication and to become tumorigenic. One paper has recently appeared in Molecular Carcinogenesis and the other is now accepted for publication in Molecular Carcinogenesis. This latter work is significant in that it now sets the stage for the development of an in vitro/in vivo model system (which, if successful, might help eliminate the need for animal testing for liver tumors). [Manuscripts, enclosed].

Most significantly, a normal rat glial line has been transformed with the neu oncogene (derived from a human neuroblastoma). These cells also had reduced cell-cell communication and when surgically inserted into the rat brain, gave rise to tumors in 3 months. Both of these in vitro oncogene-down regulated gap junction cell lines now provide the basis for developing and in vitro/in vivo system to study how chemicals, by either up regulating gap junctions will prevent cancers of the liver or brain (or other gap junction-related disease) or down regulating the gap junctions to cause the diseases. An abstract has been submitted to the upcoming Society of Toxicology meeting [see enclosed Abstract].

Most significantly, we have now shown that the "raf" oncogene, transfected into the rat liver cell line, also down-regulates cell-cell communication and causes these non-tumorigenic. We are presenting these data to the upcoming Society of Toxicology meetings and will have a manuscript for publication shortly [see enclosed Abstract].

More work on specific chemical inhibitors of these oncogene produces (i.e., Schafer et al, Science 245:379-385, 1989) could lead to therapies to

prevent certain chemical or oncogene induced cancers (or other toxic endpoints).

Aim 3. We have set up the protein kinase C assay to test if certain neurotoxicants and tumor promoters which inhibit intercellular communication in human kidney cells act by activating protein kinase C. This work is still progressing.

Aim 4. In order to continue to validate the hypothesis that chemical toxicants act by inhibiting gap junction function, we have taken a human reproductive toxicant, gossypol, and have shown it to inhibit gap junction function in rat leydig, but not rat liver cells. This shows cell type specificity of a reproductive toxicant. This paper is now in press, in Fund. Appl. Toxicol. [see enclosed].

Aim 5. As of the moment, we need antibodies to identify which of several gap junction proteins are expressed in certain cell types, as well as in normal, premalignant and malignant cells. Within the next four months, we should have two antibodies to the liver (connexion 32) and the heart (connexion 43) gap junctions. This will enable us to do phosphorylation studies by immuno precipitation of gap junctions in cells treated with chemicals which might down-regulate gap junctions by activating protein kinase C. This is now one of our major projects. Since producing and characterizing gap junction antibodies having specificity is difficult and time-consuming, it will be another 6 months before we can hope to use them for experimental purposes.

Aim 6. We now have isolated 18 mutants of the rat liver epithelial cell line which do not have gap junctional communication. These genetic mutants should be invaluable for identifying the genes that code for the structure, assembly, function and regulation of gap junctions. Two of these lines when put back into the liver gave rise to tumors. They will also be great donors for "gene therapy" by transfection with the three gap junction genes which we now have. We are in the process of putting these gap junction genes into expression vectors in order to transfect the gap junction deficient cells.

We will also start to probe these 18 mutants for the presence or absence of any or all of these gap junction gene products. A preliminary characterization of these cell lines has been submitted for publication. The publication has been provisionally accepted for publication. We are now doing an additional experiment

to satisfy the reviewers.

Recent Publications

1. J.E. Trosko and C.C. Chang, "Stem cell theory of carcinogenesis", Toxicology Letters, 49:283-295, 1989.
2. J.E. Trosko, "Towards understanding carcinogenic hazards: A crisis in paradigms", J. Amer. College of Toxicol., 8:1121-1132, 1989.
3. J.E. Trosko, C.C. Chang and B.V. Madhukar, "Cell-cell communication: Relationship of stem cells to the carcinogenic process", In: Mouse Liver Carcinogenesis: Mechanisms and Species Comparisons, D.E. Stevenson *et al.*, Eds., Alan R. Liss, Inc., New York, pp. 259-296, 1990.
4. M. El-Fouly, J.E. Trosko, C.C. Chang and S.T. Warren, "Potential role of the human Ha-ras oncogene in the inhibition of gap junctional intercellular communication", Molecular Carcinogenesis, 2:131-135, 1989.
5. J.E. Trosko, C.C. Chang and B.V. Madhukar, "In vitro analysis of modulators of intercellular communication: Implications for biologically based risk assessment models for chemical exposure", Toxicology In Vitro, in press.
6. S.Y. Oh, B.V. Madhukar, C.C. Chang, J.E. Trosko and E. Beyer, "Characterization of gap junctional communication deficient mutants from a HG-PRT deficient rat liver epithelial cell line", provisionally accepted to Exp. Cell Res.
7. A.W. de Feijter, J.S. Ray, C.M. Weghorst, J.E. Klaunig, J.I. Goodman, C.C. Chang and J.E. Trosko, "Correlation between the methylation status of the v-Ha-ras oncogene, gap junctional communication and tumorigenicity in rat liver epithelial cells", Molecular Carcinogenesis, accepted for publication.
8. B.V. Madhukar, J.E. Trosko, and C.C. Chang, "Chemical, oncogene and growth factor modulation of gap junctional communication in carcinogenesis". In: Cell Interactions and Gap Junctions. Vol. 1, N. Sperelakis and W.C. Cole, eds., CRC Press, Boca Raton, FL, pp. 143-157, 1989.

Abstracts

1. B.V. Madhukar, S.Y. Oh, E. de Feijter and J.E. Trosko, "Inhibition of intercellular communication by toxic xenobiotic chemicals in vitro in a human epithelial cell system", Soc. of Toxicology, Atlanta, GA, Feb. 27 - March 3, 1989. The Toxicologist 9:4, 1989.

2. S.G. Lilly, E. de Feijter, C.M. Weghorst, C.C. Chang, B.V. Madhukar, M. El-Fouly, J.E. Trosko, and J.E. Klaunig, "Reduced gap junctional intercellular communication and tumorigenicity of rat liver epithelial F344-WB cells". Soc. of Toxicol., Atlanta, GA, Feb. 27-March 3, 1989. The Toxicologist 9:123, 1989.
3. J.E. Trosko, "Mechanisms of chemical and oncogene modulation of gap junction and communication during carcinogenesis", Workshop on the Molecular and Cell Biology of Gap Junctions, Irsee, Germany, July 18-23, 1989.
4. J.E. Trosko, C.C. Chang, and B.V. Madhukar, "In vitro analysis of modulators of intercellular communication Implication to mechanisms of tumor promotion and to predictions of potential tumor promoters". 2nd Intern. Conf. on Practical In Vitro Toxicology, Nottingham, U.K., July 23-27, 1989.
5. J.E. Trosko, J.E. Klaunig, B.V. Madhukar, C.C. Chang, E. de Feijter, and G. Kalimi, "Chemical and oncogene modulation of intercellular communication during carcinogenesis". Symposium on Molecular Cell Biology of Liver Growth and Function, Lake Placid, NY, Aug. 13-16, 1989.
6. J.E. Trosko, J.E. Klaunig, M. Yeager, A. Koestner, M. El-Fouly, J. Buboltz, B. Cool, and C.C. Chang, "Correlation between the reduction of gap junctional communication and tumorigenesis in rat glial and liver epithelial cells containing the expressed neu oncogene". Society of Toxicology Meeting, Miami Beach, FL, Feb. 12-16, 1990.
7. A.W. de Feijter, M.W. Lieberman, and J.E. Trosko, "Up-regulation of a metallothionein-ras T24 fusion gene in rat liver epithelial cells is correlated with the down regulation of gap junction function". Society of Toxicology Meeting, Miami Beach, FL, Feb. 12-16, 1990.
8. R.J. Ruch, J.E. Trosko, B.V. Madhukar, P. Somani, and J.E. Klaunig, "Restoration of gap junction intercellular communication by lovastatin in WB-H-rasII in rat liver epithelial cells". Society of Toxicology Meeting, Miami Beach, FL, Feb. 12-16, 1990.
9. B.V. Madhukar, H. Hong Xu, B. Lockwood, and J.E. Trosko, "Inhibition of intercellular communication by environmental chemicals in rat Leydig cells in vitro". Society of Toxicology Meeting, Miami Beach, FL, Feb. 12-16, 1990.
10. W.J. Paradee, B.V. Madhukar, and J.E. Trosko, "Mezerein inhibition of intercellular communication and activation of protein kinase C in human kidney epithelial cells". Society of Toxicology Meeting, Miami Beach, FL, Feb. 12-16, 1990.

11. G. Kalimi, J.E. Trosko, L.L. Hampton, S.S. Thorgeirsson, and A.C. Huggett, "Gap junctional intercellular communication in raf and raf/myc transformed rat liver epithelial cell lines". American Association of Cancer Research Meeting, Washington, D.C., May 23-26, 1990.

Meetings Attended and Seminars Given

1. Invited seminar speaker, "Chemical modification of intercellular communication: Implications for nongenotoxic toxicity". Program in Toxicology seminar series, Rutgers University, New Jersey, March 8, 1989.
2. Invited seminar speaker, "Chemical promoters, oncogenes, and growth factor modulation of intercellular communication: A unified theory". Loma Linda, California, March 21, 1989.
3. Invited symposium speaker, "Inhibition of intercellular communication during radiation and chemical carcinogenesis. Radiation Research meeting, Seattle, Washington, March 27, 1989.
4. Invited workshop speaker, "Promotion as a factor in risk assessment". Carcinogen Risk Assessment, Society for Risk Analysis, Wash. D.C., April 4, 1989.
5. Invited symposium speaker, "Oncogenesis and abnormal intercellular communication and its implication to the cause and prevention of carcinogenesis", and "Clinical implications of intercellular communication on wound healing". Quebec Association of General Surgeons, Quebec City, Canada, May 4-5, 1989.
6. Invited seminar speaker, "Oncogenes, tumor promoters and growth factor inhibition of intercellular communication: Integrative theory of cancer". University of Hamburg, Germany, July 17, 1989.
7. Invited symposium speaker, "Mechanisms of chemical and oncogene modulation of gap junctional communication during carcinogenesis". Molecular Cell Biology of Gap Junctions Symposium, Irsee, Germany, July 18-23, 1989.
8. Invited symposium speaker, "In vitro analysis of modulators of intercellular communication: Implications to mechanisms of tumor promotion and to predictions of potential tumor promoters". Practical In Vitro Toxicology Symposium, University of Nottingham, England, July 23-27, 1989.
9. Invited symposium speaker, "Chemical and oncogene modulation of intercellular communication during carcinogenesis".

Symposium on Molecular, Cellular Biology of Liver Growth and Function, Lake Placid, NY, Aug. 13-16, 1989.

10. Invited seminar speaker, "Oncogenes, tumor promoters, and growth factors: An integrated theory of carcinogenesis". Eppley Cancer Center, Univ. of Nebraska, Omaha, Sept. 28, 1989.
11. Invited seminar speaker, "Role of chemical inhibition of gap junctional communication in pharmacological and toxicological effects of drugs". Bristol Myers, Syracuse, NY, Oct. 9, 1989.
12. Invited symposium speaker, "Modulated intercellular communication: Consequence of extracellular communication". Eicosanoids and Bioactive Lipids in Cancer and Radiation Injury, Detroit, MI, Oct. 11-14, 1989.
13. Invited seminar speaker, "Modulation of gap junctional communication: Pharmacological and toxicological implications". Park-Davis Warner Lambert Pharmaceuticals, Ann Arbor, MI, Oct. 17, 1989.
14. Invited seminar speaker, "A crisis in paradigms: The roles of genotoxic versus epigenetic mechanisms in toxicology". Environmental and Industrial Health Department, Univ. of Michigan, Ann Arbor, MI, Dec. 1, 1989.