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To: C. K. Ellis

Date: January 24, 1990

From: G. J. Patskan

Subject: Project 6906 Accomplishments for 1989

I. Phorbol Dibutyrate (PDBu) Binding

- A. A time course of binding experiment showed that 2R1 CSC decreased non-specific binding in as little as 15 minutes (1).
- B. A method to evaluate the effects of CSC at 4°C without affecting non-specific binding was developed (1).
- C. Using this method it was shown that the effects of 2R1 and X6D5RN CSCs were minimal and both CSCs produced similar effects (1).
- D. Pretreatment of the cells for 19.5 hr at 37°C with 2R1 or X6D5RN CSC produced small increases in binding with little difference between the two CSCs (1).

II. Epidermal Growth Factor (EGF) Binding

- A. There were no significant differences in the reduction of EGF binding induced by 2R1 and X6D5RN CSCs (2).
- B. The use of inhibitors of arachidonic acid metabolism (indomethacin, ETYA and NDGA) suggested that 2R1 CSC does not act through these pathways to reduce EGF binding (3).

III. Epidermal Growth Factor Receptor Phosphorylation

- A. A method to measure ATP levels in 3T3 cells was established in the lab (4).
- B. Treatment of 3T3 cells with CSC and other agents did not significantly decrease the amount of ATP per cell (5).

IV. Catechol-Depleted CSC

- A. A chromatography method was developed which would remove catechol from 2R1 CSC. This method proved to be very non-selective (6).
- B. Work was initiated to determine the feasibility of degrading catechol in CSC using the enzyme catechol oxidase (7).

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V. Glutathione Depletion Assay (GDA)

- A. Pretreatment of cells with indomethacin (a cyclooxygenase inhibitor) either increased, decreased or did not change the response 2R1 CSC depending on the experiment (8).
- B. Pretreatment of cells with NDGA (a lipoxigenase inhibitor) resulted in an inhibition of CSC's ability to reduce glutathione levels (8).

VI. Protein Kinase C in Intact Cells Assay (Protein Phosphorylation)

- A. Quiescent 3T3 cells had lower levels of basal phosphorylation than log-phase cells (9).
- B. TPA, X6D5RN CSC, and 2R1 CSC all elicited increases in protein phosphorylation. The increased phosphorylation was observed in many different proteins. The rank order of activity was TPA > X6D5RN CSC > 2R1 CSC (9).
- C. Treatment with a mixture of TPA and 2R1 CSC resulted in phosphorylation levels intermediate to those produced by each agent alone (9).
- D. Treatment with a mixture of X6D5RN CSC and 2R1 CSC resulted in phosphorylation levels greater than with either alone (9).
- E. A completion report was issued (9).

VII. JB6 Transformation Assay

- A. A literature review was completed and a research proposal was written (10-11).
- B. Dr. B. Smith was consulted regarding the conduct of the assay (12).
- C. TPA, benzoyl peroxide, mezerein and phorbol diacetate were tested in the plate-growth toxicity assay (13-15).
- D. TPA dose response experiments showed the maximal induction of colonies in soft agar to be around 2.0 - 3.5 ng/ml (13-15).
- E. An automatic colony counter was obtained (16).

References

- 1. Burruss, T. and Vaughan, B. Notebook 8804.
- 2. Stagg, D. Notebook No. 8553, pp. 184-189.
- 3. Patskan, G. J. and Stagg, D. L. The Effects of Inhibitors of Arachidonic Acid Metabolism on the Response to 2R1 CSC in the EGF Binding Assay. Memo to McCuen, R. W.; 1989 December 16.

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4. Stagg, D. Notebook No. 8883, pp. 27-31; 45-50.
5. Stagg, D. Notebook No. 8883, pp. 58-65; 67-71.
6. Vaughan, B. Notebook No. 8537, pp. 126-129; 138-143; 144-151; 163-180 and Notebook No. 8828, pp. 55-60; 80-84; 86-89.
7. Vaughan, B. Notebook No. 8828, pp. 104-109.
8. McCoy, W. Investigation of the Effect of the Release of Arachidonic Acid and Its Metabolism on the GSH Level of V79 Cells Treated with CSC. Memo to Patskan, G. J.; 1990 in preparation.
9. Nixon, G. M. Protein Kinase C Assay in Intact Cells. Special Report 89-047; 1989 September 7.
10. Nixon, G. M. Potential Promotion Assays. Memo to Penn, J. M.; 1989 April 10.
11. Nixon, G. M. Proposal for the Use of the JB6 Mouse Epidermal Cell Transformation Assay in Project 6906. Memo to Penn, J. M.; 1989 April 17.
12. Nixon, G. M. Bonita Smith's Consultation Visit Regarding JB6 Cells - July 17-18, 1989. Memo to Patskan, G. J.; 1989 July 27.
13. Nixon, G. M. Notebook 8711, pp. 124-147.
14. Vaughan, B. G. Notebook No. 8892.
15. Nixon, G. M. Notebook No. 8796.
16. Nixon, G. M. Notebook 8796, p. 159.

cc: R. W. McCuen
Project 6906

AA. Patskan

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- Bibliography -

- Patskan, G. J. Current Procedures for the PDBu Assay. Memo to Penn, J. M.; 1989 January 24.
- Penn, M. 1989 Operational Plans for the Lowered Biological Activity Program. Memo to Ellis, C. K.; 1989 February 6.
- Burruss, T. Equipment Maintenance Contracts for 1989 - Project 6906. Memo to McCuen, R.; 1989 February 14.
- Burruss, T. First Quarter Update of the LBA Program. Memo to Distribution; 1989 March 1.
- Patskan, G. J. Trip Report - 1989 Annual Meeting of the Society of Toxicology. Memo to Penn, J. M.; 1989 March 21.
- Nixon, G. M. Potential Promotion Assays. Memo to Penn, J. M.; 1989 April 10.
- Patskan, G. J. Request for Permission to Attend the Gordon Conference on Cancer. Memo to Penn, J. M.; 1989 April 14.
- Patskan, G. J. Request for Permission to Attend the GEMS Spring Meeting. Memo to Penn, J. M.; 1989 April 14.
- Nixon, G. M. Proposal for Use of the JB6 Mouse Epidermal Cell Transformation Assay in Project 6906. Memo to Penn, J. M.; 1989 April 17.
- Penn, J. M. Dr. Roger Walk's Visit. Memo to Distribution; 1989 May 8.
- Stagg, D. L. Plans for Examining CSC's Effects on BALB 3T3 Cells Using EGF Receptor Phosphorylation. Memo to Penn, J. M.; 1989 May 9.
- McCoy, W. R. Proposal: To Investigate the Effect of the Release of Arachidonic Acid and Its Metabolism on the GSH Level of V79 Cells Treated with CSC. Memo to Penn, J. M.; 1989 May 17.
- Patskan, G. J. Protocols for the Maintenance of the 3PC and MT1/2 Mouse Keratinocyte Cells Lines. Memo to Penn, J. M.; 1989 May 22.
- Burruss, T. and Penn, M. Second Quarter Update of the LBA Program. Memo to Distribution; 1989 June 1.
- Patskan, G. J. Plans to Examine the Role of Phospholipid Metabolism in the EGF Assay. Memo to Penn, J. M. 1989 June 5.
- Penn, M. Justification for the Purchase of Forma Scientific Incubators and Telephone Security System. Memo to McCuen, Dr. R. W.; 1989 June 8.
- Patskan, G. J. Trip Report: Genotoxicity and Environmental Mutagen Society (GEMS) Spring Meeting. Memo to Penn, J. M.; 1989 June 8.

2060531607

Patskan, G. and Penn, M. Record Keeping Procedures for Hazardous Chemicals in Project 6906. Memo to Distribution; 1989 July 6.

Stagg, D. TRIP REPORT: Cell Culture and Hybridomas: Quality Control and Cryopreservation Techniques. Memo to Patskan, G.; 1989 July 25.

Stagg, D. L. Suggested Studies on the Comparative Analysis of BALB/c 3T3 Clone A31 Cells Cultured in Colorado Calf Serum Versus Cells Cultured in GIBCO Calf Serum. Memo to Patskan, G. J.; 1989 July 26.

Nixon, G. M. Dr. Bonita Smith's Consultation Visit Regarding JB6 Cells - July 17-18, 1989. Memo to Patskan, G. J.; 1989 July 27.

Patskan, G. J. Operational Plans for the Lowered Biological Activity Program - 1989 Second Quarter Update. Memo to Sanders, E. B.; 1989 August 3.

Burruss, T. and Patskan, G. Relocation of Items During Labor Day Weekend Due to Shutdown of the Electrical Service. Memo to Maiden, F.; 1989 August 8.

Patskan, G. J. Survey of Laboratories for Radioisotopes. Memo to Frisch, A. F.; 1989 August 15.

Nixon, G. M. Protein Kinase C Assay in Intact Cells. Special Report 89-047; 1989 September 4.

Burruss, T. Third Quarter Update of the LBA Program. Memo to Distribution; 1989 September 7.

Patskan, G. J. Trip Report - Gordon Conference on Cancer, August 21-25, 1989. Memo to McCuen, R. W.; 1989 September 26.

Nixon, G. M. Request for Permission to Attend the Conference on Chemically-Induced Cell Proliferation: Implications for Risk Assessment. Memo to McCuen, R. W.; 1989 September 28.

Patskan, G. J. Operational Plans for the Lowered Biological Activity Program - 1989 Third Quarter Update. Memo to Sanders, E. B.; 1989 September 29.

Patskan, G. J. Annual Meeting of the American College of Toxicology. Memo to McCuen, R. W.; 1989 October 3.

Burruss, T. J. Emergency Medical Services Symposium - Trip Request. Memo to Patskan, G. J.; 1989 October 10.

Nixon, G. M. Trip Report on the Tenth Annual Meeting of the American College of Toxicology, October 30 - November 1, 1989. Memo to Patskan, G. J.; 1989 November 6.

Patskan, G. J. Trip Report - Chemically Induced Cell Proliferation: Implications for Risk Assessment. Memo to McCuen, R. W.; 1989 December 12.

2060531608

Patskan, G. J. and Stagg, D. L. The Effects of Inhibitors of Arachidonic
Acid Metabolism on the Response to 2R1 CSC in the EGF Binding Assay.
Memo to McCuen, R. W.; 1989 December 16.

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