**INTRODUCTION**

Lung cancer is the leading cause of cancer mortality for men and women in the United States. While decreasing the risk for this cancer will occur through cessation of tobacco use, lung cancer will remain a major public health problem for decades to come even if all the national smoking cessation goals were met. This is because there are many former smokers and those who have been exposed to second hand smoke. Methods are needed to treat lung cancers and prevent them in the high-risk population of ex-smokers where genetic damage to the bronchial epithelium may have occurred. All-trans-retinoic acid (RA), a derivative of vitamin A, signals growth arrest and differentiation in tumor cell lines. It also causes complete remissions in acute promyelocytic leukemia. By altering gene expression of species involved in differentiation and by affecting regulatory molecules involved in cell cycle control via interactions with nuclear retinoid receptors (RARs and RXRs), RA has been shown to reverse aerodigestive tract neoplasia in the clinic and cause growth suppression in preneoplastic cells. Further studies of the mechanisms engaged in these effects would be invaluable to overcome limitations involved in RA treatments by overcoming resistance to RA in lung carcinogenesis. To investigate RA resistance in the lung, we derived a resistant cell line designated as the BEAS-2B-R1 line from a RA sensitive bronchial epithelial cell line, BEAS-2B, by passage in 0.1 μM RA cells. To determine the mechanism of resistance, the roles of the various retinoid receptors and the closely related retinoid receptors were evaluated by Western analysis. Our findings point to suppression of the critical retinoid receptor, RARα, as the lesion responsible for retinoid resistance in human bronchial epithelial cells.

**RESULTS**

1. Establishing the RA Resistant Behavior of BEAS-2B-R1

   RA Response of Sensitive and Resistant Cell Lines

   **RA Resistant Cells Lack RAR-β Induction and No Longer Degrade G1 Cyclins in Response to RA**

   **Western Blot Protein Analysis**

   • BEAS-2B and BEAS-2B-R1 cells were plated at an equal density in a 96-well plate. After 72 hours of treatment, the samples were assayed at an equal density in a 96-well plate.

   **Cell Titer Glow Growth Assay**

   • We have found that RA induces a greater degree of growth suppression in the parental, BEAS-2B, cell line than in the RA resistant cell line, BEAS-2B-R1. This confirms the derived cell line is resistant to RA.

   • The combination of RA and 5-Aza (5AZA) shows an increase in the growth suppression of BEAS-2B-R1 cells over RA or 5AZA treatments alone (data not shown). This may be due to the restoration of RARα induction demonstrated by Western blot analysis.

2. Finding the Receptor Responsible for RA resistance and How It Can Be Overcome

   **Treatment with 5-Azacitidine Restores RARα Induction and Cyclin D1 Degradation in Resistant Cells**

   **Retinoids and Bronchial Epithelial Cells**

   How do retinoids work?

   - Retinoids and retinoids induce the cell cycle via effects on cyclin levels.
   - RA and RX Receptor Expression in Resistant Cells: Patterns Found in Lung Carcinogenesis

   **BEAS-2B-RA RAR**

   • RARα and RARγ expression appears similar in the two cell lines. These findings are reminiscent to those found in clinical lung cancer specimens and suggest that this model system may be useful for further study of how RA resistance is acquired during lung carcinogenesis.

**METHOD**

1. Growth Suppression Effect Evaluation

   - Cell Titer Glow Growth Assay
     - BEAS-2B and BEAS-2B-R1 cells were plated at an equal density in a 96-well plate.
     - Treatment consisted of a range of RA dosages as well as a vehicle treated control.
     - After 72 hours of treatment, the samples were compared using an MTT cellular growth assay to determine growth suppression.
     - In addition, the same assay was used to evaluate the growth suppression of RA, the demethylating agent, 5-azacytidine (5AZA), and the combination in BEAS-2B-R1 cells.

2. Western Blot Protein Analysis

   • Evaluated changes in protein level expression of the three RA receptors and three retinoid receptors after RA + 5AZA treatments. Aimed to identify which receptor(s) which shows restored expression that would explain the dependence on RA for growth suppression in these cells.

**CONCLUSIONS**

Growth Suppressive Effects of RA alone, 5AZA in BEAS-2B (non-RA resistant) and BEAS-2B-R1 (RA resistant) cells:

- We have found that RA induces a greater degree of growth suppression in the parental, BEAS-2B, cell line than in the RA resistant cell line, BEAS-2B-R1. This confirms the derived cell line is resistant to RA.
- The combination of RA and 5AZA shows an increase in the growth suppression of BEAS-2B-R1 cells over RA or 5AZA treatments alone (data not shown). This may be due to the restoration of RARα induction demonstrated by Western blot analysis.
- Western Blot Analysis of the Retinoid and Retinoid Receptors in BEAS-2B and BEAS-2B-R1 cells after RA and 5AZA Treatments:
  - Only RA-RAR shows a consistent increase in protein level expression after RA+5AZA treatments.