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J. Couch 2/99

PI - Signature Date

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Overview

The purpose of this award is to study the transcriptional regulation of the BRCA2 breast cancer predisposition gene with the goal of identifying agents capable of modulating BRCA2 expression.

The grant was originally awarded to the University of Pennsylvania, where early studies took place. Initially, reagents for transcriptional studies wre generated. The promoter was cloned into luciferase reporter vectors, and expression constructs of BRCA2, BRCA1, p53, p21, p27 and a number of other cell cycle regulating genes were generated. Early reporter experiments utilized these constructs in MCF-7 breast cancer cells. Transfection efficiency was normalized using a LacZ expressing pCH110 construct. Studies demonstrated that BRCA2, BRCA1, p53, p21, p27, E2F, SV40-T had no effect on BRCA2 expression. Thus BRCA2 would not seem to be directly modulated by regulators of the cell cycle.

In November 1997, I moved to the Mayo Clinic and initiated transfer of the grant. The transfer request has been considered and approved and the grant has now been awarded to the Mayo Clinic. Details of this process are available from Kathy Dunn and Kathy Smith. However, the process of transfer took approximately 14 months to complete. No financing was available at the Mayo Clinic to support this research and as a result only a minor amount of further work has been performed. Since notification of the award was made the Mayo Clinic has provided funds to re-initiate the research. Thus, some new work has been completed since December 1998, and this is outlined below.

Experimental Results

1) The specific aims of the project have not changed. No manuscripts regarding the trranscriptional regulation of BRCA2 have been published.

2) As previously reported, specific aim #1 has been completed. Mutation screening of high risk breast cancer patients for promoter mutations has failed to identify any disease associated variants.

3) Several of the previous promoter constructs were found to contain errors. These constructs were re-engineered. A number of novel constructs have also been engineered in an effort to generate a series of deletion constructs crossing the entire 7kb BRCA2 promoter. In the last two months we have generated 8 constructs which delete large regions at the 5' and 3' ends of the promoter. These constructs have been used in new reporter assays using a dual luciferase assay system (Promega) to normalize for transfection. This system is superior to the previously used LacZ system.

4) Reporter studies in MCF7 (breast) and 293T (embryonic kidney) cells have determined that the basal activity of the BRCA2 promoter is controlled by cis-acting elements in a region between 500bp 5' of the transcriptional start site (TSS) to 400bp 3' to the TSS. The region 3' to the TSS is critical for promoter activity. This region is known to contain GC boxes that bind SP1 transcription factors. Thus we will test the involvement of SP1 by observing the effects of placing inactivating mutations in the GC boxes on promoter activity. A 500bp region located 1500bp 5' of the TSS also correlates with activity in 293T cells. This region may contain a binding site for a breast or kidney specific activator of BRCA2. Further deletion studies of this region will be undertaken in order to identify the specific controlling element.

5) In order to identify the controlling elements within the 900bp basal activating region a series of 10 deletion constructs have been generated. These constructs contain the basal region with 100bp deletion from either the 5' or 3' end. No reporter assays have yet been performed.

6) Three new constructs which specifically delete the 3' region of the promoter containing putative enhancer and silencer elements are being generated. The goal is to assess the putative effect of the enhancers on BRCA2 expression.

Future Experiments

1) The 3' region of the basal promoter will be intensively studies to identify GC boxes or other sites that regulate BRCA2 expression. The involvement of critical sites will be verified by mutagenesis and electromobility shift assays.

2) The enhancer, silencer, and 5' tissue specific activator regions will be similarly studied.

3) Microdeletions of the basal activating region will be used to identify other critical regulatory elements.

4) Tissue specificity will be further studied using cell lines from other tissues such as ovary, prostate, and pancreas.

5) The effect of exogeneous agents on BRCA2 expression will be assessed. For instance, cell lines containing the promoter will be exposed to 4 Gy of gamma irradiation. The influence of radiation exposure on BRCA2 expression will be measured by Northern blot of RNA from the exposed cells and by reporter assay. Other exogeneous agents under study will include other forms of radiation including UV rays, IR rays, and chemicals that induce thymine dimers and other forms of radiation damage in DNA. Cellular mitogens such as interleukins and interferon will be tested, and finally genes such as activated Her2/neu and Ras that regulate cellular signalling mechanisms.