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Award title: LINE-1 retrotransposons as mutagens in human breast cancer.

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Original goal of the project.

It is known that the human LINE-1 retrotransposon (L1Hs) is transcriptionally active in many breast cancer cells. The original goal of the project was to test the possibility that L1Hs retrotransposition events result in the inactivation of tumor suppressor genes by insertional mutagenesis. A tagged L1Hs element was to be stably integrated into a nonmalignant breast cell line (MCF10A) and allowed to retrotranspose. Malignant cells would then be identified and the genes into which the L1Hs element had integrated could be isolated using the tag.

Summary of previously reported research.

To test the system constructs were made placing the first ORF of L1Hs under the control of various promoters (RSV, CMV, MMTV) in vectors having drug resistant genes. MCF10A cells were transfected using a variety of protocols, e.g., calcium phosphate-DNA coprecipitation, Lipofectin, etc. Although many drug resistant colonies were obtained, none expressed L1Hs. Other vectors, including retroviral vectors, were later tried, but with the same negative result. A bicistronic vector, pIRES1neo, did work, however, and gave rise to stable drug resistant colonies that expressed the initial ORF of L1Hs at high levels. This led us to hope that the complete, tagged L1Hs element could be similarly integrated into the genome and expressed using the bicistronic vector.

Summary of research not previously reported.

We constructed a vector, IRES101, containing the following elements: CMV promoter - complete L1Hs element containing a neo gene plus intervening sequence in reverse orientation (to detect retrotransposition events and serve as the tag) - intervening sequence - IRES sequence - hygromycin resistance gene - polyA signal. To see how well the construct would work it was transfected into HeLa cells and various combinations of, (a) time before drug treatment and, (b) TPA treatment (a phorbol ester known to enhance transcription from the CMV promoter) were tested. Retrotransposition events could be detected in a number of experiments, showing that the construct was functional. However, the numbers of retrotransposition events were orders of magnitude lower than those needed to have a realistic chance of inactivating tumor suppressor genes. In an attempt to boost the number of retrotransposition events the IRES101 vector has been modified in several ways, but these vectors have not yet been tested. Cell lines developed during the course of the project, which express L1Hs proteins under the control of the CMV promoter, may prove useful in studying the interaction(s) of L1Hs proteins with other cellular proteins.

Publications/manuscripts.

No publications or manuscripts have yet come out of this work.