Cyclin E — a better prognostic marker for breast cancer than cyclin D?

To the editor — In their recent article, Patricia Steeg and colleagues report that overexpression of cyclin D mRNA occurs in most invasive ductal cancers of the breast, distinguishing invasive breast cancers from nonmalignant lesions[1]. While the findings pertaining to cyclin D mRNA overexpression are timely and important, there was no mention of the prognostic role of another G1 cyclin, namely cyclin E. It was also suggested that cyclin D1, but not E, might function as the growth-limiting restriction-point protein (R protein). We would like to take this opportunity to call attention to the relevant properties of cyclin E, emphasizing its importance in prognosis of early stages of breast cancer and to propose cyclin E as a better candidate for the R protein.

We have documented that altered expression of cyclin E may be associated with breast cancer[2]. Using normal proliferating breast cells versus human tumor breast cells as a model system, we observed a number of alterations in cyclin E expression, including an eightfold amplification of the cyclin E gene in one tumor cell line and aberrant expression in all ten tumor cell lines examined. The deranged production of cyclin E in tumor cells is quantitative and qualitative as cyclin E protein is severely overexpressed in tumor cells and present in lower molecular weight isoforms not observed in normal cells.

We have also extended these observations to the *in vivo* situation by examining the pattern of cyclin E protein expression in tumor and normal adjacent tissues obtained from breast cancer patients[3]. We found that the altered expression of cyclin E protein occurred in most of the breast tumor tissues examined, that the alterations increased with increasing grade and stage of the tumor, and that these alterations were more consistent than c-erb B2 or cyclin D1 overexpression in breast cancer. Furthermore, cyclin E was also altered in other types of solid tumors as well as leukemia. Collectively these observations suggest that the altered expression of cyclin E in the breast tumor samples is not a mere consequence of cell proliferation but represents a significant difference between normal tissue and low- and high-stage tumors and, as such, represents a potential new prognostic marker for breast cancer[3]. Recently we have further extended these studies. We have examined 400 new breast tumor specimens and compared the changes of cyclin E expression with seven other tumor markers, and have shown that cyclin E protein is the most consistent marker for determining the prognosis of early-stage node-negative ductal carcinomas (K.K., manuscript in preparation).

Others have corroborated our findings and demonstrated that immunocytochemical detection of cyclin E detects tumor proliferation and deregulated cyclin expression[4]. The mechanism of the cyclin E alteration is in part a result of its deregulation in breast cancer. Recently, we have documented that while cyclin E protein and its associated kinase activity in normal mammary epithelial cells are cell-cycle regulated, in tumor cells it remains in an active complex throughout the cell cycle[5].

In addition to its role as a prognostic indicator for breast cancer, cyclin E also may function as the R protein. We have proposed three properties to characterize the R protein, as derived from cell biology experiments with mouse 3T3 cells[6].

In cell biological experiments, cycloheximide applied during G1 inhibits total protein synthesis. During a several hour pulse the R protein is lost in normal cells, as a consequence of its instabiltiy. Its resynthesis requires time, and so transit to S phase of these cells is delayed. In contrast, the stable or overproduced R protein in a tumor cell is not degraded and additional delay is not observed[6].

Similarly, pulse-chase experiments were performed in which cyclins E and A and their related kinase activities were measured[7]. By the above criteria, either E or A cyclins could be the R protein. We have recently repeated this experiment with cyclin D and showed that the results were quite different, in that cyclin D protein in both normal and tumor cells disappeared and recovered equally and rapidly with no extra delay of recovery. Therefore, cyclin E (or A) fits our criteria for the R point better than does cyclin D.

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Steeg and colleagues reply — We would like to thank Dou, Pardee and Keyomarsi for their informative letter. Our article addressed the mRNA levels of cyclins A and D in premalignant lesions and early carcinomas of the breast. Certainly other cyclins, and other proteins, may contribute to malignant progression. Whether cyclin D or E functions as a "restriction-point protein" is a question that we did not specifically address. We noted the correlation of cyclin D overexpression with any form of carcinoma, and speculated that it may serve proliferative or nonproliferative functions. Given the complexity of cancer development and progression in virtually any cell type studied, it is likely that multiple genetic events are required, and both cyclins D and E may be significant influences.

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