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Using a molecular titration assay to quantitate and characterize the c-erbB2 mRNA synthesized in human mammary adenocarcinoma cell lines.

The human *c-erbB2* gene is a member of a family of growth factor receptor genes encoding cellular membrane proteins structurally related to the EGF receptor (KRAUS *et al.*, 1989). The c-erbB2 protein is detected in normal kidney and mammary human cells (GULLICK *et al.*, 1987; BENZ *et al.*, 1989). The amplification of the c-erbB2 gene and the overexpression of the c-erbB2 protein are observed in 30% of the human primary breast and ovarian cancers (SLAMON *et al.*, 1989) and few other adenocarcinomas (gastric and salivary gland, colon) with the exclusion of other types of malignancies (CALLAHAN & CAMPBELL, 1989). The epithelial cell-specific effect of the *c-erbB2* oncogene is illustrated by studies of transgenic mice carrying the activated *c-neu* oncogene (the rat gene homolog to *c-erbB2*) under the transcriptional control of the MMTV-LTR (MULLER *et al.*, 1988; BOUCHARD *et al.*, 1989). In these animals, the expression of the activated *c-neu* is a necessary factor acting as a predisposing event to induce the malignant transformation of the mammary gland (BOUCHARD *et al.*, 1989).

We characterized five human mammary adenocarcinoma cell lines for the synthesis of the c-erbB2 mRNA (Northern- and dot-blot analyses, RNase protection assays) and for the presence of amplified copies of the *c-erbB2* gene (Southern- and dot-blot analyses). The c-erbB2 mRNA levels measured in two cell lines (HBL100 and MCF-7) are comparable with that found in the normal human adult kidney. Two other cell lines (T47-D and MDA-MB453) overexpress different c-erbB2 mRNA levels in the absence of gene amplification. The highest c-erbB2 mRNA level is measured in the BT474 cells which contain multiple copies of the *c-erbB2* gene.

In vivo, similar classifications were established between the breast tumors investigated for the status of the *c-erbB2* gene (SLAMON *et al.*, 1989). In any case, we did not find aberrantly sized c-erbB2 mRNA.

As already discussed (KRAUS *et al.*, 1987), the discrepancy between the measured c-erbB2 mRNA levels and the *c-erbB2* gene dosage suggests that the overexpression of the c-erbB2 protein in human mammary tumor cells can be caused by different molecular mechanisms. In order to precise these molecular abnormalities, we first searched for possible modifications of the c-erbB2 mRNA stability in the different tumor cell lines. Using single-stranded RNA probes in solution hybridization reactions, we performed molecular titration assays (LEE & COSTLOW, 1987) to quantitone the c-erbB2 mRNA content in the different cell lines and to monitor the c-erbB2 mRNA degradation in the same cells treated with actinomycin-D under conditions where the protein synthesis is inhibited or not. These results are currently analysed.

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