were equivalent to those in MDA-MB-468 and T47D cells, which were reported to contain PTEN loss and a *PIK3CA* hotspot mutation without *HER2* amplification, respectively [23]. These findings therefore indicated that *HER2* amplification itself may have equivalent biological effect on PI3K signaling with PTEN loss or *PIK3CA* hotspot mutation. In addition, our results are consistent with a recent study by Oda et al. [33], in which they showed that HER2 and/or HER3 overexpression, PTEN, or *PIK3CA* mutations occur almost exclusively in breast and other cancer cell lines.

Findings in past and present studies may potentially lead to beneficial clinical applications. For *HER2*-amplified breast cancer showing no *PIK3CA* mutations, trastuzumab is likely to be effective, with possible rescue using HER2-TKIs in cases of relapse. For *HER2*-amplified breast cancer with *PIK3CA* mutations, inhibitors against molecules of the PI3K pathway are possibly more effective than anti-HER2 agents, which are unlikely to be beneficial.

In addition to pharmacogenetic approaches, including *PIK3CA* genotyping, pharmacodynamic markers are potentially powerful tools in individualized use of molecularly targeted therapy. In a number of previous pharmacodynamic studies on HER2- or EGFR-targeted therapy, phospho-Akt was used as a surrogate marker for PI3K pathway activity [34, 35]. In the present study, however, growth inhibition is more closely associated with changes in phospho-S6K than that in phospho-Akt. These findings indicate that the prediction of tumor response to trastuzumab may strongly benefit from measurements of S6K phosphorylation levels. The cause of the discrepancy between the association of cell growth with phospho-Akt and that with phospho-S6K, however, remains unclear. It may be due to the difference in sensitivity of phospho-specific antibodies used in the present study or the higher sensitivity of phospho-Akt to positive feedback signals following initial inhibition of the PI3K pathway compared with phospho-S6K.

The present study shows several limitations. First, although a relatively large panel of *HER2*-amplified breast cancer cell lines ($N = 8$) were used, the properties of all HER2-overexpressing breast tumors are not necessarily represented. Despite HER2 amplification being retained, particular tumor subtypes may have been selected in the establishment of cell lines. Secondly, in addition to inhibition of HER2 signaling, a few studies have indicated the contribution of antigen-dependent cellular cytotoxicity (ADCC) in the antitumor effect of trastuzumab. Because ADCC only works in *in vivo* conditions, our current data do not necessarily deny the potential effect of trastuzumab on tumors showing *PIK3CA* mutations [36]. Thirdly, although wild-type *PIK3CA* appeared necessary for trastuzumab sensitivity *in vitro*, other factors may be involved, as shown by results showing moderate resistance of HCC1419 to trastuzumab (Figure 2C). The mechanisms of *PIK3CA*-unrelated resistance remain unknown but are under current investigation in our laboratory.

In conclusion, our findings show an association between the presence of *PIK3CA* hotspot mutations and resistance to not only trastuzumab but also HER2-TKI in naturally derived *HER2*-amplified breast cancer cell lines. Further, PI3K inhibitors are potentially effective in overcoming trastuzumab resistance caused by *PIK3CA* mutations. Assessment of S6K phosphorylation levels may be a useful pharmacodynamic marker correlated to the antitumor effect of HER2-targeted therapy. A better understanding of these findings, however, may require further investigation in clinical trials and concomitant translational studies.

funding

Grant-in-Aid for Young Scientists (B) from Ministry of Education, Culture, Sports, Science and Technology of Japan to T.M.; AstraZeneca Research Grant 2007 to T.M.; Kobe University Medical School Research Grant for Young Scientists to T.M.; Grants-in-Aid for Cancer Research from Ministry of Health, Labor and Welfare of Japan to H.M.

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