Annals of Oncology **12** *(Suppl. 1)*: S21–S22, 2001.

Symposium article

Modulation of p27/Cdk2 complex formation through 4D5-mediated inhibition of HER2 receptor signaling

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Summary

The molecular mechanisms mediating the anti-proliferative effects of the murine anti-HER2 monoclonal antibody (4D5) were investigated in HER2-overexpressing human carcinoma cell lines. Treatment with 4D5 resulted in a dramatic accumulation of BT-474 breast carcinoma cells in G1; concomitant with reduced expression of proteins involved in sequestration of the cyclin E/Cdk2 inhibitor protein p27, increased association of p27 with Cdk2 complexes and Cdk2 inactivation. No equivalent effects were observed in BT-474 cells treated with a control, non-inhibitory HER2 monoclonal antibody (FRP5) or in a HER2-overexpressing cell line insensitive to 4D5 treatment (MKN7 gastric carcinoma cells), confirming the relationship between these molecular changes and 4D5-mediated inhibition of proliferation. Increased p27 expression was also observed in 4D5-treated BT-474 cells; however an antisense approach demonstrated that this increase was not required for Cdk2 inactivation or establishment of the G1 block. These data suggest that 4D5 interferes with HER2 receptor signaling, resulting in downregulation of proteins involved in p27 sequestration. This causes release of p27, allowing binding and inhibition of cyclin E/Cdk2 complexes and inhibition of G1/S progression. This model was confirmed using a second 4D5-sensitive, HER2-overexpressing breast tumor line (SKBR3), and suggests that the dependency of a given tumor cell on elevated HER2-receptor signaling for the maintenance of p27 sequestration proteins may determine the clinical response to treatment with the humanized anti-HER2 monoclonal antibody Herceptin® (trastuzumab).

Key words: 4D5 antibody, cell cycle, HER2 overexpression, Herceptin, p27, trastuzumab

In a mammalian somatic cell, cell cycle progression is regulated by the sequential activation of a family of protein kinases, known as the cyclin-dependent kinases (Cdks). In association with specific regulatory cyclin subunits, these kinases play a major role in determining whether cells proceed through each cell cycle phase or make the transition from one cell cycle phase into the next. One Cdk which plays a major role in determining whether cells enter S phase and duplicate their DNA is cyclin E/Cdk2. Together with cyclin D-dependent Cdks, cyclin E/Cdk2 is involved in phosphorylating and inactivating the growth-suppressor protein retinoblastoma. This results in derepression of transcription factors that modulate the expression of genes required for S phase entry and progression. Due to the major role this kinase plays at the G1/S transition, the activation state of cyclin E/Cdk2 is stringently regulated not only by phosphorylation/dephosphorylation events, but also through association with a small inhibitory protein (p27). In order for cells to go through the G1/S transition, p27 is made unavailable for binding to cyclin E/Cdk2 through complex formation with p27-sequestration proteins such as the cyclin D-dependent Cdks. Additionally, p27 is targeted for degradation via ubiquitin-mediated proteolysis, an event requiring phosphorylation of p27 by cyclin E/Cdk2 itself.

The anti-proliferative effects of the murine anti-HER2 monoclonal antibody (4D5) on HER2-overexpressing tumor cell lines have been reported previously [1–3] and, in ovarian carcinoma cells, this has been correlated with increased p27 levels [4]. However, the exact effect of 4D5 on cell cycle modulators has not been fully elucidated until now (see Lane et al. [5]). Researchers at the Friedrich Miescher Institute have compared the effects of 4D5 with that of a non-inhibitory control antibody, FRP5, which also binds to the extracellular domain of HER2. The BT-474 breast tumor cell line was chosen for this study because it is known to be exquisitely sensitive to 4D5 treatment [2, 3]. BT-474 cells were exposed to either FRP5 or 4D5 for 48 hours, or remained untreated. Subsequent analysis by flow cytometry demonstrated that control and untreated cells displayed a normal cell cycle profile, whereas 4D5 treatment resulted in almost total accumulation in the G1 phase, suggesting a block in G1/S progression. Consistent with this G1 block, retinoblastoma protein was shown to be in its hypophosphorylated, growth inhibitory form, and cell cycle markers of S phase as well as G2/M were absent. Most strikingly, however, a large increase in p27 protein expression was observed, which correlated with increased association of p27 with Cdk2 and with Cdk2 inactivation.

From these data, it was initially assumed that 4D5 was affecting HER2 receptor signaling, resulting in an increase in p27, which then inhibited Cdk2 kinase and, hence, prevented the G1/S transition. However, more detailed kinetics showed that p27 started to accumulate only after eight hours 4D5 treatment, whereas cyclin E/Cdk2 inactivation was evident after two hours. Moreover, by eight hours, Cdk2 activity was already reduced to 50% of that found in control cells. Further investigation revealed that p27 started to move onto Cdk2 complexes after two hours, where it subsequently accumulated. This movement of p27 was shown to correlate with the loss of proteins involved in p27 sequestration, and decreased association of p27 protein with cyclin D/Cdk4 complexes. The relationship between these molecular changes and inhibition of proliferation was confirmed by repeating the analysis with a HER2-overexpressing cell line unaffected by 4D5 treatment (MKN7 gastric carcinoma cells). As expected, in MKN7 cells no recruitment of p27 onto Cdk2 complexes occurred following 4D5 treatment. Additionally, no equivalent effects on the expression of proteins involved in p27 sequestration were observed.

The next stage was to determine whether increased p27 protein levels are involved in establishing the G1 block in BT-474 cells, or whether the movement of p27 onto Cdk2 complexes is sufficient. In order to address this question, increased p27 protein expression in response to 4D5 treatment was prevented using a p27 antisense approach. As expected, after 24 and 36 hours of 4D5 treatment, control cells exhibited an increase in p27 protein expression. In contrast, no increase in p27 levels was seen in antisense-treated cells. Despite this, antisense-treated cells were found to block in G1 after 36 hours to a similar extent as observed in control cells. This indicated that increased p27 protein expression is not required for establishing the 4D5-induced G1 block in BT-474 cells. Moreover, further analysis did indeed reveal that Cdk2 was inactivated in p27 antisense-treated cells. This occurred a little more slowly than in control cells, but by 36 hours Cdk2 was almost totally inactivated, as in the controls. This correlated with a shift of p27 protein onto Cdk2 complexes in both control and antisense-treated cells.

Based on these data, a model of how 4D5 inhibits proliferation in HER2-overexpressing cell lines was proposed as follows. Treatment with 4D5 interferes with HER2 receptor signaling, causing downregulation of proteins involved in p27 sequestration. This results in the release of p27, allowing it to bind and inhibit cyclin E/Cdk2 complexes. Without this activity the cell cannot go through the G1/S transition, thereby delaying or even halting cell cycle progression. An increase in p27 protein expression also occurs in BT-474 cells. However, from the antisense approach as well as p27 half-life studies, this was postulated to be a consequence of cyclin E/Cdk2 inactivation leading to inefficient targeting of p27 to the proteosome degradation machinery. This model has been confirmed through analysis of a second 4D5-sensitive, HER2-overexpressing breast tumor cell line (SKBR3).

It was further speculated that the dependency of a given tumor cell on elevated HER2 receptor signaling for the maintenance of p27 sequestration proteins may determine the clinical response to treatment with the humanized anti-HER2 monoclonal antibody trastuzumab (Herceptin). Future research, therefore, should focus on elucidating what determines whether a cell becomes dependent on HER2 overexpression during tumorigenesis, and the relative contribution of other HER2 receptors to this phenomenon.

Acknowledgements

This work was supported in part by a grant from the Swiss Cancer League to H.A. Lane. A. B. Motoyama acknowledges support from the Stipendium Kommission für Nachwuchskräfte aus Entwicklungsländern, Basel-Stadt, Switzerland.

Note

The authors have not reported any financial relationships with companies whose products are mentioned in the text.

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