Controlled Internalization and Recycling of Her-2/neu by Cross-linking with an Avidin/streptavidin-biotin System for MR Enhancement

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Introduction

Avidin/streptavidin-biotin system is frequently used in pretargeting approaches to link antibody and targeting agent together due to the rapid reaction between avidin/streptavidin and biotin with high affinity. In the ideal pretargeting setting, cell membrane bound biotinylated antibody is tagged by avidin, which subsequently binds to a biotinylated therapeutic or imaging agent. However, avidin is a tetramer that can bind to four biotin molecules. Therefore, avidin can link more than one biotinylated antibody together, which can result in extensive cross-linking of the surface bound biotinylated antibody before binding it to the final target agents. We show here the internalization and recycling of the Her-2/*neu* receptors induced by the above cross-linking and its application in the MR signal enhancement.

Material and methods

Her-2/*neu* positive BT-474 human mammary carcinoma cells were grown according to the ATCC protocols. About 10^4 cells were seeded in removable 4-chambered glass slides for confocal microscopy studies. Cells were treated with biotinylated Herceptin for 0.5 hour, washed, treated with streptavdin-TexasRed 0.25 hour, washed again, incubated for 3.5 hours, and treated with biotinylated Herceptin 0.5 hour, washed, and treated with streptavidin-488 0.25 hour. Cells were then washed and fixed with 3% paraformaldehyde before being imaged with a Zeiss LSM 410 microscopy. About 10^7 were harvested and treated in the same way as above but with biotinylated Herceptin and avidin-(DTPA-Gd) for MR image studies with a 9.4T Bruker AVAVCE spectrometer.

Results and discussion

Herceptin itself is resistant to internalization, Figure 1A. However, when the surface Her-2/*neu* bound biotinylated Herceptin bind to avidin, it is internalized in less than 3.5 hrs, Figure 1B. During the same time period, Her-2/*neu* receptor returned to the surface, as shown in Figure 1C.

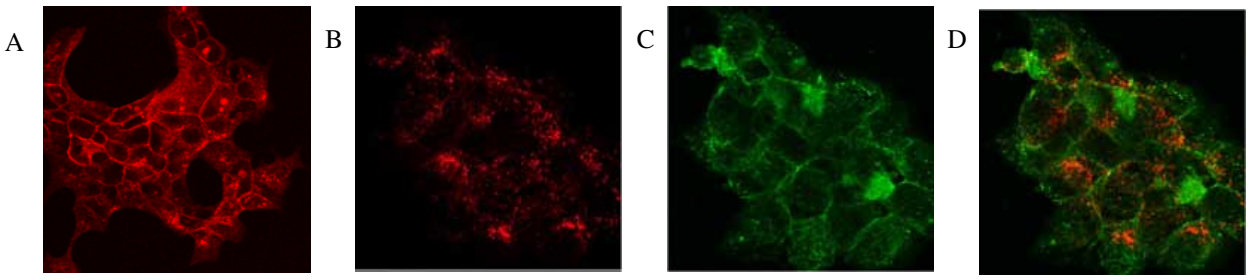


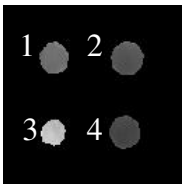
Figure 1. Confocal images of BT-474 cells. A. At 4 hours after treatment with Herceptin-Alexa680, B. and C. BT-474 treated with biotinylated Herceptin, streptavdin-TexasRed, biotinylated Herceptin, and streptavdin-488 as describe above. D. overlay of B. and C.

We think the avidin/streptavidin-biotin system induced internalization of Herceptin is due to the extensive cross-linking of surface bound biotinylated Herceptin by avidin. As shown in Figure 2, the four biotin binding sites afforded by tetramer avidin can produce an extensive 2-D lattice of biotinylated Herceptin-avidin complex on cell membrane and this complex is subsequently internalized.

Figure 2. A graphic illustration of 1-D crosslinking of antibody by avidin/streptavidin-biotin system, an extensive 2-D lattice can form on cell surface, following the same pattern with the two remaining binding sites of avidin.

◇ avidin receptors ○ biotin Y antibody

Since Her-2/*neu* receptors are recovered 3.5 hours after being internalized, we tried delivering an MR contrast agent, avidin-(Gd-DTPA) conjugate repeatedly following a two-step pretargeting approach. BT-474 cells treated with biotinylated Herceptin and avidin-(Gd-DTPA) twice 3.5 hours apart showed additional decrease in T1 values comparing to that with single treatment. The results are shown in Figure 3.



1..BT-474 cells T1= 2.2s
2. BT-474 1X T1=1.8s
3. BT-474 2X T1=1.4s
4. Water T1=3.8s

Figure 3. T1 map. 1. untreated BT-474 cells, 2. BT-474 cells treated with biotinylated Herceptin and avidin-(Gd-DTPA) once, 3. water, and 4. BT-474 cells treated with biotinylated Herceptin and avidin-(Gd-DTPA) twice 3.5 hrs apart.

Conclusion

We can control the internalization of Herceptin by cross-linking it with an Avidin/streptavidin-biotin system. Her-2/*neu* receptors recycled back to surface 3.5 hours after being internalized. We can increase the loading of MR agent by repeated delivery, utilizing the "internalization-recycle" path.

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