PEPTalk
January 11 - 15, 2010, Coronado, CA

EGFR-Specific DARPins as Potential Cancer Therapeutics

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Members of the epidermal growth factor receptor (EGFR) family have been implicated in the development and progression of many tumours. Monoclonal antibodies directed against EGFR and HER2 are currently approved in cancer therapies. However, they need to be used in conjunction with chemotherapy, by themselves the clinical efficacy is limited; this would make new formats necessary. Designed ankyrin repeat proteins (DARPins) may overcome these limitations since multivalent, multispecific or conjugated proteins can be produced much more easily than with antibodies or other protein scaffolds, realistically offering new treatment routes.

Previously, four DARPins with nanomolar affinities were selected on the EGFR ectodomain in vitro via phage display. They were shown to bind to A431 cells overexpressing EGFR in flow cytometry analysis. Binding of selected DARPins was confirmed by fluorescence microscopy. The effect of selected DARPins on A431 cells was examined in a number of cell-based assays. DARPins E01, E67 and E68 affected cell proliferation (clonogenic assay), cell viability (XTT assay) and cell cycle (G1 arrest) in a similar manner as cetuximab. The effect on cell proliferation was even stronger for the DARPins than for cetuximab. DARPin E69 showed no biological effect on A431 cells. DARPin Off7, selected on maltose binding protein (MBP), was used as a negative control, showing that the effect of E01, E67 and E68 was indeed specific. Furthermore, DARPins E01, E67 and E68 had an inhibitory effect on the phosphorylation of EGFR, ERK, and AKT. In the case of phosphorylation of AKT, the effect of selected DARPins was even better than that of cetuximab.

The epitopes of DARPins E01, E68 and E69 were mapped by investigating antigen-binding using yeast-surface display of EGFR. The epitopes of E01 and E68 overlapped with the epitope of cetuximab on domain III, whereas E69 bound to an epitope located on domain II. Similar to cetuximab, E01 and E68 interfered with binding of EGF to EGFR, thus inhibiting receptor activation.

In conclusion, it can be said that DARPins can have a therapeutic effect on tumour cells, similar to, even more pronounced than conventional antibodies such as cetuximab. In addition to these biological effects, DARPins can get internalised, thereby extending the therapeutic application of DARPins.