INVESTIGATION OF THE BINDING SPECIFICITY OF IGF-1R USING MONOCLONAL ANTIBODIES

By

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Abstract

The insulin-like growth factor type one receptor (IGF-1R) plays a critical role in cancer. The receptor is over expressed in many tumours and mediates growth, motility, and survival from apoptosis. Several studies have shown that the inhibition of IGF-1R's expression or its function blocks tumour growth or metastasis, and also enhances sensitivity to cytotoxic drugs and irradiation. To date, IGF-1R is widely considered as a very promising target for cancer treatment.

Among different techniques for targeting IGF-1R, blocking the receptor with specific monoclonal antibodies (MAbs) is an attractive way to inhibit the receptor signalling. In addition, antibodies specific for the IGF-1R are potential diagnostic reagents as the IGF-1R is overexpressed in several cancers. A similar approach has been successfully utilized in the treatment of breast cancer using Herceptin, which is a humanised MAb against human epidermal growth factor receptor-2.

In this thesis, two high affinity murine MAbs against IGF-1R namely 7C2 and 9E11 were isolated and cloned. To obtain these MAbs, s-IGF-1R (the soluble extracellular part of the IGF-1R, amino acids 1-906) was used as an immunogen. These MAbs were IgG1κ isotype and did not cross react with either insulin receptor isoform IR-A or IR-B. MAbs 7C2 and 9E11 successfully detected IGF-1R in a number of immunoassays such as in enzyme linked immunosorbent assays (ELISAs), flow cytometry and immunohistochemistry. The MAbs also immunoprecipitated IGF-1R from lysates of cells overexpressing recombinant IGF-1R. Both MAbs 7C2 and 9E11 showed high affinity to the s-IGF-1R. The binding affinities of the MAbs 7C2 and 9E11 to s-IGF-1R were 0.5±1.6 nM and 2.1±0.4 nM, respectively. The sequences of the variable regions of these MAbs' heavy and light chains were also described in this study. Comparison of the CDR sequences of the MAbs with those of other MAbs against the IGF-1R showed that MAbs 7C2 and 9E11 each had a unique sequence.

During this research it was found that MAbs 7C2 and 9E11 blocked the binding of IGF-I to the IGF-1R, but did not show an inhibitory effect on the binding of IGF-II to this receptor. Moreover, epitope mapping of MAbs 7C2 and 9E11 revealed that they both bound to the cysteine-rich domain of the receptor. These findings support a previous study, which demonstrated that the cysteine-rich domain of IGF-1R is critical for specific binding of IGF-I but not IGF-II to the receptor.

In this thesis, further epitope mapping studies on MAbs 7C2 and 9E11 using alanine mutants of the IGF-1R in the cysteine-rich domain are reported. The results showed that amino acids F241, F251 and F266 were involved in binding to both MAbs 7C2 and 9E11. On the other hand, another study showed that amino acids F241 and F251 were crucial for IGF-I binding to the receptor. Therefore, binding to these two amino acids by MAbs 7C2 and 9E11 is most likely responsible for the selective blocking of IGF-I but not IGF-II to the receptor.

Competition experiments performed during this research revealed that the chimeric IGF analogues IGF-ICII (IGF-I with IGF-II C domain) and IGF-IICI (IGF-II with IGF-I C domain) behaved like IGF-II and IGF-I, respectively in their ability to inhibit the binding of MAbs 7C2 and 9E11 to s-IGF-1R. These findings imply that out of all four domains of IGF-I, it is the C domain, which binds to residues in IGF-1R that form the epitope for MAbs 7C2 and 9E11. Because in the C domain of IGF-I, amino acids R36 and R37 and also Y31 have been shown to be important for binding to IGF-1R, it could be proposed that the binding of the IGF-I C domain to the IGF-1R cysteine-rich domain is through binding amino acids R36, R37 and Y31 of IGF-I to residues F241 and F251, or to the nearby residues, which are sterically affected by the presence of the MAbs binding to the receptor.

In this research, *in vitro* studies, using MAbs as anti-cancer reagents revealed that both MAbs 7C2 and 9E11 inhibited the proliferation of colon cancer cell (HT-29) induced by IGF-I and IGF-II. However, the neutralizing effect for IGF-II was less potent than for IGF-I. Since the MAbs did not inhibit binding of IGF-II to the receptor, their effect on IGF-II induced cell proliferation could be claimed to be due to an indirect effect. This is consistent

with the *in vitro* result for the receptor down-regulation experiment, which showed that 24 h incubation of breast cancer cells (MCF-7) with MAbs 7C2 and 9E11, significantly reduced the IGF-1R expression. In this study it was also shown that MAbs 7C2 and 9E11 inhibited MCF-7 cells migration induced by IGF-I. This could indicate a potential anti-metastatic property of MAbs 7C2 and 9E11.

Taken together, the *in vitro* effects of MAbs 7C2 and 9E11 reported in this thesis revealed that they had inhibiting effects on cancer cell proliferation and migration and they also induced IGF-1R down-regulation. Therefore, they could be potentially valuable prospects for cancer treatment if humanised.

Finally, MAbs 7C2 and 9E11 could be used as diagnostic reagents to detect IGF-1R in cells or tissue samples in a range of immunoassays as mentioned earlier. Due to the IGF-1R overexpression in several cancer tumours, MAbs 7C2 and 9E11 could be utilized for malignant tumour recognition. Furthermore, as another application, these MAbs could also be used to identify tumours overexpressing the IGF-1R, allowing tailored anti-cancer treatment including an IGF-1R blocking agent.