P-170 Peptides with low similarity to the human proteome: tracing an effective and safe biological way towards effective and safe cancer chemotherapy

Darja Kanduc1,*, Giuseppe Novello1 and Roberto Mazzanti2

1Department of Biochemistry and Molecular Biology “Ernesto Quagliariello”, University of Bari, Italy and 2Department of Internal Medicine, University of Florence, AOU Careggi, Florence, Italy

Correspondence to: D. Kanduc, Department of Biochemistry & Molecular Biology, University of Bari, Italy. Telephone: 390805403321 E-mail: d.kanduc@biologia.uniba.it, dkanduc@gmail.com

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We propose low-similarity P-170 peptide-based antibodies to neutralize the multidrug resistance phenomenon and, consequently, improve the treatment regimens for cancer chemotherapy. As a first step in the experimental validation of this approach, we report on the similarity analysis of the P-170 primary amino acid structure versus the human proteome and describe peptide motifs uniquely owned by the human P-170 glycoprotein.

Key words: P-170, MDR, cancer chemotherapy, drug resistance, vaccines, low-similarity peptides

INTRODUCTION

The multi-drug-resistance (MDR) phenomenon is a main leading cause of chemotherapy failure in the treatment of neoplastic disease (1). MDR is associated with an overexpression of the human multi-drug-resistance gene mdr1, which encodes an energy-dependent transmembrane 170kD glycoprotein, also known as P-170. One biological function of P-170 is to transport some chemotherapeutic agents out of cells, thereby conferring a drug resistant phenotype to cancer cells expressing P-170 (1–3). There are several cancers in human pathology where MDR phenotype is involved: leukemias, lymphomas, myelomas, breast and ovarian cancers (4, 5). In addition, non-small-cell-lung carcinoma, colon, liver and renal cancers, malignant bone and soft tissue tumors are relatively refractory to chemotherapy (6–9). Given the incidence of these cancer diseases (10), MDR phenomenon is a potential obstacle to the effective treatment of about 85% of cancer patients.

Chemical agents such as channel blockers (verapamil), calmodulin antagonists, triparanol analogs, quinidine, cyclosporine, etc. (11–14) have been proposed in order to overcome or circumvent P-170 mediated multi-drug-resistance. However, the clinical use of these chemosensitizers has been hampered by serum protein binding and/or toxic side effects that occur when non-physiological doses, which are required to achieve a significant reversal of MDR, are used (11, 14, 15). Therefore, in spite of the intensive and massive efforts in numerous laboratories worldwide, so far none of the proposed approaches has been useful for overcoming the MDR phenomenon. Multi-drug-resistance in cancer remains an unsolved problem (16–19).

In addition, occurrence of the MDR phenotype has prognostic negative value as it is quite likely that overexpression of P-170 in plasma membrane is associated with other characteristics of cancer cells that confer them a more aggressive behavior (20, 21). For what briefly reported, it would be most probably important to circumvent the MDR phenomenon in cancer patients to ameliorate patient’s prognosis.

Among the approaches aimed to reverse the MDR phenomenon, antibodies specific to the P-170 glycoprotein have been developed. The data obtained are encouraging and suggest that the induction of auto-antibodies to P-170 might effectively reverse MDR in cancer chemotherapy (22–24). However, these immune treatments are not exempt from side effects. As an example, granulomas, possibly due to autoimmune cross-reactions, have been found in the pancreas, adrenals, spleen, and ovaries of mice following immunization (22, 24). This observation, combined
with previous reports on cross-reactivity of anti-P-170 antibody with p185 c-erbB2 (25), invites to caution. Indeed, targeting tumor cells is only one of vaccine’s requirements. In addition, a vaccine has to be safe and void of adverse side-effects. In conflict to these unavoidable prerequisites, as a matter of fact, the current antigen-specific immunotherapeutic protocols target not only the antigen-positive cells, but also normal tissues expressing other antigens but sharing sequences with the target antigen (26).

In this context, our laboratory is developing peptide-based immunotherapies based on the low-similarity hypothesis (27, 28). The low-similarity hypothesis defines the immune unit as a sequence with no/low similarity to the host proteome. Consequentially, the hypothesis explains the non-immunogenicity of tumor-associated antigens as possibly due to a high level of similarity of oncprotein sequences to the self proteome while assigns immunogenic potential to peptides characterized by a low similarity to the host proteome (i.e., to amino acid sequences that are not present in host proteins). In the course of the last decade, we experimentally validated the low-similarity concept using autoimmune and cancer disease models (29–42). Moreover, we demonstrated a possible therapeutic utilization of low-similarity peptides (42). Indeed, the first and most important application of the low-similarity hypothesis regards the design and development of safe and effective vaccines devoid of cross-reactivity. Vaccine preparations based on no-similarity antigen segments are expected to specifically hit the antigen overproduced and uniquely expressed by the tumor, without harmful side cross-reactions with other proteins.

The present study examines the possibility of developing specific and safe antibodies against portions of P-170 with no counterpart in the human proteome. The final goal would be to abolish MDR P-170 over-expression in cancer cells or, at least, its effect(s) on cell biology, therefore making practicable, effective and safe anti-cancer chemotherapies currently in clinical use.

This idea appears intriguing for several reasons: first, its simplicity. Antibodies against specific peptide fragments uniquely belonging to the P-170 protein would specifically hit the MDR-associated protein without collateral harmful damage. Second, the concrete possibility of reaching an otherwise "unthinkable" goal: usage of chemotherapeutic regimens at concentrations well below the toxicity level. Indeed, neutralization of the counter-acting P170 protein would eliminate the necessity of increasing chemotherapy dosages in order "to push" the chemical reagents inside the cancer cell. Third, the possibility of reaching parts of the human body that are considered so far like "sanctuaries" for anticancer drugs, unreachable for the majority of them, such as the brain. Useless to say, patients’ advantages both as life quality and time survival would be incredibly high.

METHODS

Pentapeptide similarity analysis of the human P-170 protein was conducted against the human proteome using Protein Information Resource (PIR) database (http://pir.georgetown.edu/pirwww) (27–42). In brief, the human P-170 protein (accession number UniProtKB/Swiss-Prot P08183, also known as ATP-binding cassette sub-family B member 1 or CD antigen 243) primary amino acid sequence was dissected into pentamer motifs that were used as probes to scan the human proteome searching for exact identical matches using PIR perfect Peptide Match Program. The pentamer probes were offset by one residue: i.e., MDLEG, DLEGD, LEGDR, EGDRN, etc. The P-170 protein was removed from the human proteome when the sequence similarity analysis was done. The similarity level of a P-170 pentamer to the human proteome is calculated as the number of times the P-170 pentamer occurs in the set of ~35,000 proteins that comprehensively form the human proteome. The similarity level is zero when the P-170 5-mer is absent in the human proteome.

RESULTS

The literature indicates 5 to 6 amino acids as sufficient minimal antigenic determinants critically involved in immune recognition (43, 44). Consequently, P-170 pentapeptide probes were used for proteome scanning. The 170kD glycoprotein is formed by 1280 amino acids for a total of 1276 pentapeptides sequentially overlapped by four residues. Searching for pentapeptide(s) representing unique molecular signature of the P-170 protein, each of the P-170 1276 pentapeptides was analyzed for similarity to the human proteome. The results of the similarity analysis are reported in Table 1.

As a first note, we observe that only 42 pentapeptides out of total 1276 ones are identity signatures exclusively owned by the P-170 glycoprotein. Scientifically, this datum indicates that about 97% of P-170 5-mers are shared with other human proteins. In the immune context, this means that a vaccine formulation using the entire P-170 protein as an antigen would produce a 97% risk of possible cross-reactivity with human proteins. It
P-170 peptides with low similarity to the human proteome

has also to be underlined that this percentage actually underestimates the effective risk since it does not consider the pentapeptide redundancy level, i.e. the number of times each shared 5-mer from the p-170 protein is repeated in the whole human proteome. Therefore it appears quite reasonable to conclude that an anti-P-170 vaccine based on P-170 peptides with zero-similarity to the human proteome would offer a high level of effectiveness both in specificity, i.e. specifically targeting the MDR-associated protein, and safety, i.e. assuring a zero cross-reactivity risk.

A second consideration emerging from the data reported in Table 1 is that P-170 hosts also 6-mer stretches formed by two consecutive zero-similarity 5-mer. Specifically, the fragments FSMFRY, TGFFMN, AIMRQE, ERYKN, PVSPWFR, KFEHMQ, and IHAFIE appear of interest since, potentially, the use of longer unique fragments in evoking specific anti-P-170 antibodies would enhance the immune specificity.

DISCUSSION

Elsewhere we have already illustrated the potential advantages and the safety of biological therapies based on low-similarity peptide sequences (28). Here, the proposal of neutralizing the multi-drug-resistance phenomenon by non-cross reactive anti-P-170 antibodies presents an additional therapeutic value when considering the current clinical problems in chemotherapy. Indeed the MDR phenomenon, either intrinsic or acquired, leads to continuous chemotherapeutic dose escalation or, at least, association of more than one drug: the more resistant the cancer cells, the more aggressive the applied chemotherapeutic regimen.

On the other hand, the cytotoxic, mutagenic and immunosuppressing combinations are carriers of collateral effects to normal organs and physiological functions, so diminishing the quality of life as well as the patients’ survival. Indeed, the side effects of chemotherapy are generally heavy and numerous. To cite only a few of them, they may comprehend: anaphylaxis, blood clots, cardiotoxicity, deep vein thrombosis, liver dysfunction, central neurotoxicity, myelosuppression. In general, the most part of these toxic effects are due to the high drug doses needed to counteract the P-170 (over)expression. Several anticancer drugs are substrates of P-170, included many of the new drugs such as the tyrosine kinase inhibitors (47). Thus, also these drugs are subject to the cleaning up effect of P-170. Therefore, lowering the expression or the activity of P-170 offers the possibility of reducing anticancer drug doses without losing, but rather enhancing, the chemotherapy effectiveness.

In this context, the biological approach proposed in this study holds the promise of breaking the chemotherapy/multi-drug-resistance/high-dose chemotherapy vicious cycle since effective neutralization of the P-170 glycoprotein might allow least toxic and mutagenic drug regimens. In addition, P-170 is present also in cell organelles that are involved in inducing apoptosis.

Table 1. The P-170 primary sequence versus the human proteome: the unique P-170 identity spots

<table>
<thead>
<tr>
<th>Pos(^a)</th>
<th>5-mer(^b)</th>
<th>Pos(^a)</th>
<th>5-mer(^b)</th>
<th>Pos(^a)</th>
<th>5-mer(^b)</th>
<th>Pos(^a)</th>
<th>5-mer(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>FSMFR</td>
<td>162</td>
<td>WFDVH</td>
<td>694</td>
<td>PVSFW</td>
<td>945</td>
<td>TQAMM</td>
</tr>
<tr>
<td>38</td>
<td>SMFRY</td>
<td>189</td>
<td>KIGMF</td>
<td>695</td>
<td>VSFWR</td>
<td>947</td>
<td>AMMYF</td>
</tr>
<tr>
<td>41</td>
<td>RYSNW</td>
<td>192</td>
<td>MFFQS</td>
<td>698</td>
<td>WRIMK</td>
<td>983</td>
<td>FGAMA</td>
</tr>
<tr>
<td>47</td>
<td>DKLYM</td>
<td>194</td>
<td>FQSMMA</td>
<td>739</td>
<td>FTRID</td>
<td>1007</td>
<td>HIIMI</td>
</tr>
<tr>
<td>98</td>
<td>INDTG</td>
<td>208</td>
<td>FTRGW</td>
<td>789</td>
<td>RYMVF</td>
<td>1010</td>
<td>MIIEK</td>
</tr>
<tr>
<td>101</td>
<td>TGFFM</td>
<td>275</td>
<td>ERYNK</td>
<td>801</td>
<td>VSWFD</td>
<td>1012</td>
<td>IEKTP</td>
</tr>
<tr>
<td>102</td>
<td>GFFMN</td>
<td>276</td>
<td>RYNKN</td>
<td>803</td>
<td>WFDDP</td>
<td>1025</td>
<td>GLMPN</td>
</tr>
<tr>
<td>133</td>
<td>VSFWC</td>
<td>326</td>
<td>YSIGQ</td>
<td>915</td>
<td>KFEHM</td>
<td>1154</td>
<td>IHAFI</td>
</tr>
<tr>
<td>141</td>
<td>GRSQIH</td>
<td>369</td>
<td>IDNKP</td>
<td>916</td>
<td>FEHMY</td>
<td>1155</td>
<td>HAFIE</td>
</tr>
<tr>
<td>154</td>
<td>AIMRQE</td>
<td>518</td>
<td>HKFDT</td>
<td>918</td>
<td>HMYAQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>155</td>
<td>IMRQE</td>
<td>608</td>
<td>EKGNH</td>
<td>936</td>
<td>HIFGI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The P-170 pentapeptides with zero similarity to the human proteome are sequentially listed by amino acid position along the P-170 primary sequence. *Amino acid position in the P-170 protein. **Amino acid sequence of the zero-similarity pentapeptides.
Since apoptosis can be recovered by certain drugs, i.e. celecoxib (20), it is possible to suggest that antibodies against P-170 could be also used to induce apoptosis by the MDR positive cells, reverting resistance to susceptibility to anticancer drugs.

In conclusion, the data and the rationale offered in this study clearly show that the currently occurring chemotherapy problems are not insurmountable. Combining a safe biological therapy and a safe chemical regimen might be a resolutive step in the war against cancer. From a patients’ perspective, managing chemotherapy side effects would signify a remarkable positive impact on response rate, long-term survival and quality of life.

AUTHORS’ CONTRIBUTIONS

GN helped in computational analyses. RM discussed the paper and contributed to paper writing. DK proposed the original idea, designed the computational analysis, interpreted the data and wrote the paper.

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P-170 peptides with low similarity to the human proteome


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