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Structural Organization of 6B9 Molecule, a Monoclonal Antibody Against Lycopene

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Full cDNA and corresponding amino acid (AA) sequences of 6B9 monoclonal antibody (mAb) against lycopene was obtained using Step-Out RACE technology. Variable (V) and constant (C) regions were identified. The light chain of 6B9 contained 238 AA IgM with the highest level of identity (0.93) to both the anti-VEGF receptor antibody and anti-collagen type II FAb CIIC1. The heavy chain was composed of 634 AA with a high identity (0.9) to the Ig mu chain C region. Potential posttranslational modification regions in both chains were identified alongside with disulfide bond sites. The obtained information can be used for making chimeric constructs containing 6B9 mAb (or its fragments) and lycopene, a powerful carotenoid with antioxidant as well as antiproliferating properties, which can be implemented in the treatment of an aggressive form of prostate cancer and possibly other malignancies.

Keywords: monoclonal antibody, molecular structure, immunotherapy, lycopene

Introduction

Lycopene is a linear tetraterpene formed by eight condensed isoprene units and contains 11 aligned hydrogen bonds.[1] The presence of hydrogen bonds confers unique antioxidant properties to lycopene, which exceed the antiradical potential of alpha-tocopherol and carotene multiple times, helping to explain the widespread attention given by many researchers to the biological role of lycopene in modern redox medicine.[2] Lycopene cannot be synthesized in the human body[3] and needs to be ingested from lycopene-containing food products, such as tomatoes, papaya, watermelon, and some others.[4] The structural variability of the lycopene molecule predetermines the existence of various lycopene isomers. Plants contain predominantly all-*trans*-lycopene isomers.[1,2] However, physical factors (heating, supercritical CO_2 exposure, prooxidizing factors) can induce reisomerization of lycopene and formation of *cis*-isoforms.[5] Lycopene present in the human body is represented by a balance of *cis*- and *trans*- isomers.[1,4]

As mentioned, the high antioxidative potential of lycopene has attracted the attention of many researchers in recent years. Although lycopene is classed as a nonessential micronutrient and there is no recommended value for daily intake as yet, it has been proclaimed recently that lycopene deficiency could be harmful to human health status and leads to increased risk of cardiovascular disease and cancer.[6,7] Decreased levels of lycopene in serum and/or plasma specimens have been reported in aging individuals as well as in cardiovascular disease, metabolic, and neurological disorders.[7] On the other hand, lycopene supplementation has been shown to improve health status parameters in some normal physiological and pathological conditions.[8–11]

Despite the growing body of epidemiological and experimental evidence relating to the pivotal role of lycopene in human health and disease, there is no standardized protocol for lycopene measurement in biological specimens. The existing methods for lycopene quantification include highly elaborate and expensive high-performance liquid chromatography methods as well as mass spectrometry for identification of lycopene isomers.[12,13]

Recently,[14] we have developed monoclonal antibodies against lycopene which work in different biological systems and specimens in terms of quantitative and semiquantitative assays. In the present article, we report on the amino acid sequence of the newly developed monoclonal antibody against lycopene and discuss the possibilities for its use.

Materials and Methods

Generation of monoclonal antibody against lycopene

Monoclonal antibodies against lycopene were raised as previously described.[14] Briefly, lycopene gold conjugate was used for immunization of mice. Lycopene was dissolved in EtOH (0.5 mg/mL) and the solution was heated until the crystals were dissolved. The colloidal gold solution was mixed with lycopene–EtOH at a ratio of 5:1 (v/v) and kept at

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room temperature for 20 minutes. The conjugated lycopene (t-LC-gold conjugate) obtained was immediately used for injections. Suspensions of t-LC-gold conjugates were mixed with complete Freund's adjuvant and injected intraperitoneally with a 3-week interval between the first and second injections and a 2-week interval between remaining injections of conjugates. Serum samples from the immunized mice were routinely screened using indirect ELISA. After 72 hours following the final intravenous boost, the spleens of the mice (positive responders) were removed and dispersed and a fusion protocol with Sp-2 cells was performed. Positive clones were subcloned by limiting dilution. Antibodies were isotyped with a mouse monoclonal antibody isotyping reagent (Sigma-Aldrich). Finally, a positive clone 6B9, which worked most consistently in different assays, was established and deposited at the Institute of Genetics and Selection of Industrial Microorganisms (Moscow, Russia).

RNA extraction

The hybridoma cells were washed three times with ice-cold PBS by resuspension and centrifugation (4°C at 3000 *g*) and lysed with 1.0 mL of TRIzol Reagent (Invitrogen) by passing through syringe and needle. The lysed cells were allowed to remain at room temperature for 5 minutes, after which 0.2 mL of chloroform was added to the tubes and the specimens were vortexed for 1 minute. The samples were centrifuged at 14,000 g for 10 minutes at 4°C and the upper phase was carefully transferred to new tubes for further analysis. The total RNA was precipitated by addition of 0.5 mL of isopropyl alcohol and the tubes were incubated at −20°C for 15 minutes. The specimens were then centrifuged again (10,000 *g* for 10 minutes at 4°C). The supernatant was discarded and the pellet washed with 70% ethanol then dissolved spectrophotometrically in 0.05 mL of DEPC-treated water. Total RNA concentration was measured.

cDNA preparation

Synthesis of the first strand of cDNA from total RNA was performed using the MINT cDNA Kit (Evrogen) in accordance with manufacturer's instructions. Briefly, 1 μg of preheated RNA specimens were mixed with 3 μL of water, 1 μL of primer, and 1 μL PlugOligo adapter, overlaid with mineral oil, and incubated at 70°C for 2 minutes. Samples were then cooled down to 42°C and mixed with the second part of the reaction mixture containing revertase. cDNA synthesis was performed at 42°C.

Step-out RACE

The full cDNA sequence for light and heavy chains of IgM against lycopene was obtained by implementation of Step-out RACE Technology (Mint RACE cDNA amplification set; Evrogen). For the sequencing of the heavy chain coding regions three different specific primers were used: adapter primer-5′-AAGCAGTGGTATCAACGCAGAGT as well as IgM specific primers—IgM-synt1 5′-ACAACACTGAAGT CATCCAG and IgM synth 3′-PCR 5′-ACGAGGGGGAA GACATTTGG. To sequence the light chain coding regions it was first necessary to determine the class of the constant region. PCR with light chain class-specific primers demonstrated that both clones were kappa class. Then the same adapter primer and kappa class-specific primer mIGKC/out-R 5′-CATTCCTGTTGAAGCTCTTG were used for Step-Out RACE. A detailed description of the protocol for Step-Out RACE is given elsewhere.[15]

Sequence analysis

Sequence analysis was performed using the Lasergene 7 software package and Serial Cloner 2.6.1 (Massachusetts). Protein structure prediction was performed with ProteinPredict software.[16] Potential disulfide bonds were identified with the DiANNA algorithm.[17]

Results

Table 1 shows the full amino acid (AA) sequence of both the light (238 AA) and heavy (634 AA) chains of the lycopene-specific monoclonal antibody 6B9 with variable (V-region, highlighted in light gray) and constant (C-region,

Table 1. Full Amino Acid Sequence of 6B9 Light and Heavy Chains

Light chain 6B9
METDTLLLWVLLLSAWVPGSTGNIVLTQSPALASVSLGQRATISCRASESVDNYGKSFMHWYQQKPGQPPRLLIY LASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNNEDPPTFGGGTRLEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEAT HKTSTSPIVKSFNRN
Heavy chain 6B9
MNFGLSLIFLALILKGVQCEVQLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLEWVATISSGGSY TYYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHPGWGAWFAYWGQGTLVTVSAESQSFPNVFP LVSCESPLSDKNLVAMGCLARDFLPSTISFTWNYQNNTEVIQGIRTFPTLRTGGAYLATSQVLLSPKSILEGSDE YLVCKIHYGGKNRDLHVPIPAVAEMNPNVNVFVPPRDGFSGPAPRKSKLICEATNFTPKPITVSWLKDGKLVES GFTTDPVTIENKGSTPQTYKVISTLTISEIDWLNLNVYTCRVDHRGLTFLKNVSSTCAASPSTDILTFTIPPSFADIF LSKSANLTCLVSNLATYETLNISWASQSGEPLETKIKIMESHPNGTFSAKGVASVCVEDWNNRKEFVCTVTHRD LPSPQKKFISKPNEVHKHPPAVYLLPPAREQLNLRESATVTCLVKGFSPADISVQWLQRGQLLPQEKYVTPHLV TERTVDKSTGKPTLYNVSLIMSDTGGTCYEGEVNAEEEGFENLWTTASTFIVLFLLSLFYSTTVTLFKVKSAPM PEPGAPGFYFTHSILTVTEEEWNSGETYTCVVGHEAL

Total RNA was extracted from the hybridoma clone 6B9 and full cDNA and amino acid sequences were obtained as described in the ''Materials and Methods'' section. ''V'' region is highlighted in light gray, ''C'' region is highlighted in dark gray.

Table 2. Disulfide Bonds in 6B9 Heavy Chain

AA topography	*Matching sequences*
19–354	LKGVQCEVQLV–NVSSTCAASPS
41–115	SLKLSCAASGF–TAMYYCARHPG
116–443	LVAMGCLARDF–RKEFVCTVTHR
227–337	DEYLVCKIHYG–LNVYTCRVDHR
274–431	KSKLICEATNF–GVASVCVEDWN
384–627	SANLTCLVSNL–GETYTCVVGHE
491–551	SATVTCLVKGF–DTGGTCYEGEV

Total RNA was extracted from the hybridoma clone 6B9; full cDNA and amino acid sequences were obtained as described in the ''Materials and Methods'' section.

Cysteine residues involved in the formation of disulfide bonds in the heavy chain of 6B9 are underlined.

highlighted in dark gray). Figure 1 shows the AA composition pattern of both molecules. Serine, threonine, and leucine were the most abundant AA in the structure of the light chain with proportions of 12.6%, 9.4%, and 8.4%, respectively. Histidine, methionine, and cysteine formed the minority of AA in the light chain (proportions between 1.2% and 1.6%). The predominant AA in the heavy chain of IgM 6B9 was serine (9.9%) followed by leucine (9.6%) and threonine (9.3%). Methionine, histidine, and tryptophan were represented at a much smaller percentage (1.4%–2.0%).

Figure 2 shows the overall alignment of the 6B9 IgM light and heavy chain molecules. The amino terminus in both molecules starts with short protein-binding signal (AA 1–2, defined with red rhomb) followed by a helical region. This region was slightly larger in the light chain (AA 4–20, shown beneath the scale as a brown segment) as compared with the heavy chain (AA 4–15). The remaining part of both molecules contains multiple stranded regions (shown in blue). Moreover, there is a region with potential polynucleotide-binding capacity in the light chain of IgM (AA 42–46, shown by flags with yellow circles), which was absent in the structure of the heavy chain. This region was followed by three protein-binding regions—between AA 53–68, 112–128, and 212–214 in the light chain of IgM (shown in the Figure 2 by flags with red rhombs). Overall, the heavy chain contains almost the same number of potential protein-binding sites, mostly associated with the amino terminal area.

Blast search analysis showed that the light chain sequence of 6B9 IgM has the highest level of identity (0.93) to both the anti-VEGF receptor antibody and anti-collagen type II FAb CIIC1 as reported by others.[18,19] On the other hand, the heavy chain of 6B9 IgM has a high identity (0.9) to the Ig mu chain from C region.[20]

Analysis of the molecular structure of the light chain of IgM 6B9 also allowed us to verify potential disulfide bond sites. A favorable neighboring AA environment explains the high probability of disulfide bond formation between AA residues 45–114 (RATISCRASES-AATYYCQQNNE, with probability of 0.75) and between AA residues 160–220 (GASVVCFLNNF-HNSYTCEATHK, with an even higher probability of 0.95). These residues can participate in the formation of native intramolecular and extramolecular disulfide bonds and can be used for the creation of recombinant constructs containing the light chain of 6B9 IgM. The heavy chain of 6B9 IgM contains a larger number of high probability disulfide bonds as shown in Table 2.

In addition, unmatched cysteine abundantly present in the structure of light and heavy chain IgM is known to be highly susceptible to chemical modification, which can promote generation of further structural variants and analogs such as IgM constructs with trisulfide bonds or thioether linkages.[21,22]

Glutamate is another important amino acid for posttranslational modification. Polyglutamylation is a reversible modification of antibody originating from the successive covalent attachment of glutamic acid (up to 20 residues) to an internal glutamate residue of the heavy and light chains of immunoglobulins leading to changes in antibody affinity and other biological properties of Ig molecules.[23,24] Polyglutamate regions were identified in the light chain of 6B9 between residues AA 124–127 and at AA 154–155.

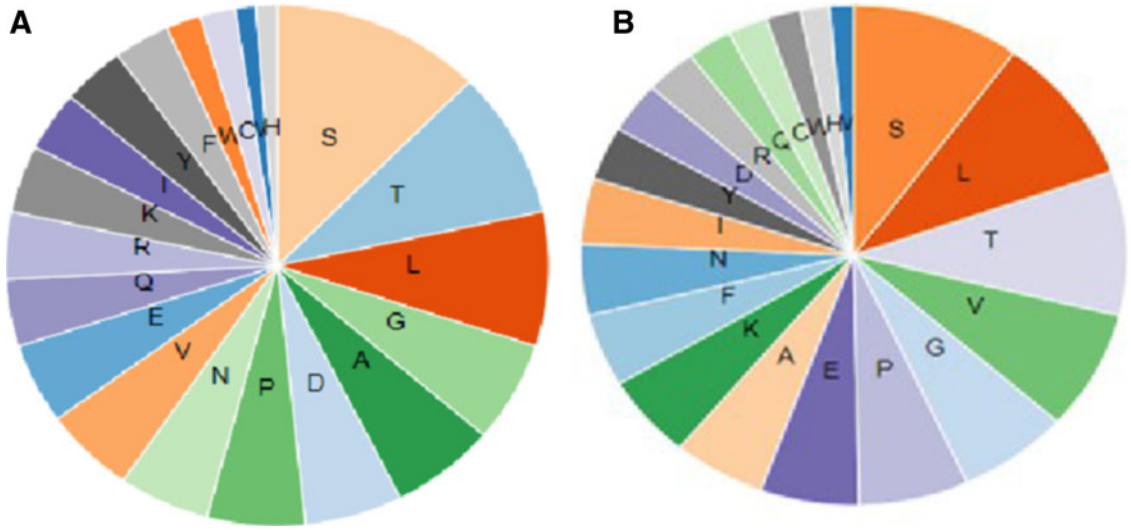

FIG. 1. Amino acid composition of light **(A)** and heavy **(B)** chains of 6B9 IgM. Total RNA was extracted from the hybridoma clone 6B9; full cDNA and amino acid sequences were obtained as described in the ''Materials and Methods'' section. Round square diagram shows representation of individual amino acids in sequences of the light and heavy chains of 6B9 IgM.

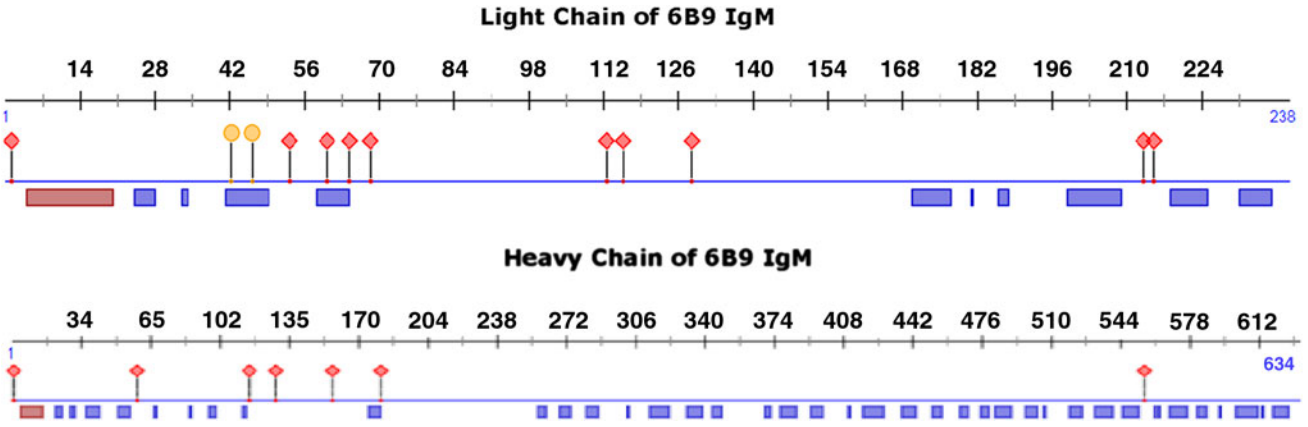

FIG. 2. Linear alignment of 6B9 IgM light and heavy chains. Total RNA was extracted from the hybridoma clone 6B9; full cDNA and amino acid sequences were obtained as described in the ''Materials and Methods'' section. Sequence analysis of the light and heavy chains of 6B9 IgM was performed using ''PredictProtein'' software.

The heavy chain of 6B9 is characterized by more abundant representation of glutamate. In particular, glutamate-rich regions were located between residues AA 120–131 and AA 548–604. The latter contained seven glutamate residues.

Additional studies are required to identify other important posttranslational modification sites, including deamidation of asparagine, and several forms of N-linked glycosylation. These sites could also be useful for chimeric construction.

Discussion

The sequencing and cloning of immunoglobulin genes is the first and often success-limiting step in the design and preparation of chimeric and/or modified immunotherapeutic antibodies. According to the results shown above, we have succeeded in preparing mRNA and amplified cDNA from hybridoma clones and obtained the sequences of heavy chain and light chain genes for immunoglobulin M 6B9 monoclonal antibody (mAb) against lycopene. The information obtained is essential for preparing immunotherapeutic and artificially designed antibody–ligand complexes, which can be constructed with lycopene-specific antibodies and/or their fragments.

Lycopene is a carotenoid, phytochemical, with a low intestinal absorption rate and low ability to pass through biological membranes.[2,7] The mechanisms behind transmembrane transport of lycopene remain poorly understood and require detailed investigation. Lycopene is known[25] to be distributed around the organs and tissues of the human body by lipoproteins (low-density lipoprotein [LDL] and very low-density lipoproteins) and to pass through membranes of hepatocytes and other LDL receptor-expressing cells through mechanisms of receptor-mediated uptake. However, cells and tissues with low expression of LDL-receptor or structural elements separated from the systemic circulation by histo–hematic barriers are deprived of lycopene influx mediated by lipoprotein transport and uptake.[7] Most tumor cells are known to have abnormal regulation of LDL receptor expression and are subject to constant antioxidant deprivation.[26,27] Reportedly, there is a complete loss of regulation of the LDL receptor expression in androgen-independent prostate cancer.[28]

On the other hand, multiple experimental studies suggest that antioxidants and lycopene in particular, are extremely potent antineoplastic agents and are capable of inhibiting cancerous cell growth under *in vitro* conditions.[29] Such an effect was observed and reported in numerous cell lines, including prostate cancer cells, brain tumors, and lung cancer cells.[30,31] However, multiple attempts to use lycopene treatment in a clinical setting have produced negative or highly questionable results,[32] which can be explained by poor bioavailability and low penetration capability of lycopene in cell membranes of cancer cells. Moreover, lycopene intake by cancer cells might be limited by glycoprotein P, which is a major gatekeeper for xenobiotic entry to neoplastic cells.[33,34]

It is a feasible and attractive prospect to develop chimeric lycopene antibody conjugated to lycopene, which can be used for delivery of lycopene to tumor cells through a receptor-mediated pathway and bypassing, thereby, interaction with glycoprotein P. These chimeric constructs could be highly effective for the treatment and reduction of tumor growth in highly aggressive forms of prostate cancer and brain tumors. Conjugation of lycopene to the whole mAb 6B9 molecule or its fragments can be done by exploiting the intrinsic affinity of mAb 6B9 for lycopene or by coupling mAb 6B9 to lycopene through different functional sites in the 6B9 molecule reported above, including free thiol groups generated by either partial reduction methods or cysteine residues in the antibody sequence. Implementation of lycopene chimeric constructs composed of lycopene and mAb 6B9 could open new possibilities in the treatment of cancer.

Author Disclosure Statement

No competing financial interests exist.

References

1. Milani A, Basirnejad M, Shahbazi S, and Bolhassani A: Carotenoids: Biochemistry, pharmacology and treatment. Br J Pharmacol 2017;174:1290–1324.
2. Saini RK, Nile SH, and Park SW: Carotenoids from fruits and vegetables: Chemistry, analysis, occurrence, bioavailability and biological activities. Food Res Int 2015;76 (Pt 3):735–750.
3. Papaioannou EH, Liakopoulou-Kyriakides M, and Karabelas AJ: Natural origin lycopene and its ''Green'' downstream processing. Crit Rev Food Sci Nutr 2016;56:686–709.
4. Khoo HE, Prasad KN, Kong KW, Jiang Y, and Ismail A: Carotenoids and their isomers: Color pigments in fruits and vegetables. Molecules 2011;16:1710–1738.

5. Xianquan S, Shi J, Kakuda Y, and Yueming J: Stability of lycopene during food processing and storage. J Med Food 2005 Winter;8:413–422.
6. Alok S, Jain SK, Verma A, Kumar M, Mahor A, and Sabharwal M: Herbal antioxidant in clinical practice: A review. Asian Pac J Trop Biomed 2014;4:78–84.
7. Petyaev IM. Lycopene deficiency in ageing and cardiovascular disease. Oxid Med Cell Longev 2016;2016:3218605.
8. Cicero AFG, Fogacci F, and Colletti A: Food and plant bioactives for reducing cardiometabolic disease risk: An evidence based approach. Food Funct 2017;8:2076–2088.
9. Aghajanpour M, Nazer MR, Obeidavi Z, Akbari M, Ezati P, and Kor NM: Functional foods and their role in cancer prevention and health promotion: A comprehensive review. Am J Cancer Res. 2017;7:740–769.
10. Roohbakhsh A, Karimi G, and Iranshahi M: Carotenoids in the treatment of diabetes mellitus and its complications: A mechanistic review. Biomed Pharmacother 2017;91:31–42.
11. Perdana NR, Mochtar CA, Umbas R, and Hamid AR: The risk factors of prostate cancer and its prevention: Acta Med Indones 2016;48:228–238.
12. Tamburini E, Costa S, Rugiero I, Pedrini P, and Marchetti MG: Quantification of lycopene, β-carotene, and total soluble solids in intact red-flesh watermelon (*Citrullus lanatus*) using on-line near-infrared spectroscopy. Sensors (Basel) 2017;17:746–758.
13. Irakli M, Chatzopoulou P, Kadoglidou K, and Tsivelika N: Optimization and development of a high-performance liquid chromatography method for the simultaneous determination of vitamin E and carotenoids in tomato fruits. J Sep Sci 2016;39:3348–3356.
14. Tsibezov VV, Bashmakov YK, Pristenskiy DV, Zigangirova NA, Kostina LV, Chalyk NE, Kozlov AY, Morgunova EY, Chernyshova MP, Lozbiakova MV, Kyle NH, and Petyaev IM: Generation and application of monoclonal antibody against lycopene. Monoclon Antib Immunodiagn Immunother 2017;36:62–67.
15. Matz MV, Alieva NO, Chenchik A, and Lukyanov S: Amplification of cDNA ends using PCR suppression effect and step-out PCR. Methods Mol Biol 2003;221:41–49.
16. Yachdav G, Kloppmann E, Kajan L, Hecht M, Goldberg T, Hamp T, Hönigschmid P, Schafferhans A, Roos M, Bernhofer M, Richter L, Ashkenazy H, Punta M, Schlessinger A, Bromberg Y, Schneider R, Vriend G, Sander C, Ben-Tal N, and Rost B: PredictProtein—An open resource for online prediction of protein structural and functional features. Nucleic Acids Res 2014;42(Web Server issue):W337–W343.
17. Passerini A, Punta M, Ceroni A, Rost B, and Frasconi P: Identifying cysteines and histidines in transition-metal-binding sites using support vector machines and neural networks. Proteins 2006;65:305–316.
18. Uysal H, Sehnert B, Nandakumar KS, Böiers U, Burkhardt H, Holmdahl R, and Thunnissen MM: The crystal structure of the pathogenic collagen type II-specific mouse monoclonal antibody CIIC1 Fab: Structure to function analysis. Mol Immunol 2008;45:2196–2204.
19. Franklin MC, Navarro EC, Wang Y, Patel S, Singh P, Zhang Y, Persaud K, Bari A, Griffith H, Shen L, Balderes P, and Kussie P: The structural basis for the function of two anti-VEGF receptor 2 antibodies. Structure 2011;19:1097–1107.
20. Kehry M, Sibley C, Fuhrman J, Schilling J, and Hood LE: Amino acid sequence of a mouse immunoglobulin mu chain. Proc Natl Acad Sci U S A 1979;76:2932–2936.
21. Moritz B, and Stracke JO: Assessment of disulfide and hinge modifications in monoclonal antibodies. Electrophoresis 2017;38:769–785.
22. Liu H, and May K: Disulfide bond structures of IgG molecules: Structural variations, chemical modifications and possible impacts to stability and biological function. MAbs 2012;4:17–23.
23. Janke C, Rogowski K, and van Dijk J: Polyglutamylation: A fine-regulator of protein function? 'Protein Modifications: beyond the usual suspects' review series. EMBO Rep 2008;9:636–641.
24. Behrens CR, and Liu B: Methods for site-specific drug conjugation to antibodies. MAbs 2014;6:46–53.
25. Moran NE, Erdman JW Jr, and Clinton SK: Complex interactions between dietary and genetic factors impact lycopene metabolism and distribution. Arch Biochem Biophys 2013;539:171–180.
26. Chen Y, and Hughes-Fulford M: Human prostate cancer cells lack feedback regulation of low-density lipoprotein receptor and its regulator, SREBP2. Int J Cancer 2001;91:41–45.
27. Vasseur S, and Guillaumond F: LDL receptor: An open route to feed pancreatic tumor cells. Mol Cell Oncol 2016;3:e1033586.
28. Furuya Y, Sekine Y, Kato H, Miyazawa Y, Koike H, and Suzuki K: Low-density lipoprotein receptors play an important role in the inhibition of prostate cancer cell proliferation by statins. Prostate Int 2016;4:56–60.
29. Raza MH, Siraj S, Arshad A, Waheed U, Aldakheel F, Alduraywish S, and Arshad M: ROS-modulated therapeutic approaches in cancer treatment. J Cancer Res Clin Oncol 2017. DOI: 10.1007/s00432-017-2464-9. [Epub ahead of print].
30. Farzaei MH, Bahramsoltani R, and Rahimi R: Phytochemicals as adjunctive with conventional anticancer therapies. Curr Pharm Des 2016;22:4201–4218.
31. Ono M, Takeshima M, and Nakano S: Mechanism of the anticancer effect of lycopene (tetraterpenoids). Enzymes 2015;37:139–166.
32. Kavanaugh CJ, Trumbo PR, and Ellwood KC: The U.S. Food and Drug Administration's evidence-based review for qualified health claims: Tomatoes, lycopene, and cancer. J Natl Cancer Inst 2007;99:1074–1085.
33. Molnár J, Engi H, Hohmann J, Molnár P, Deli J, Wesolowska O, Michalak K, and Wang Q: Reversal of multidrug resistance by natural substances from plants. Curr Top Med Chem 2010;10:1757–1768.
34. Efferth T, and Volm M: Multiple resistance to carcinogens and xenobiotics: P-glycoproteins as universal detoxifiers. Arch Toxicol 2017;91:2515–2538.

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