分子表面を覆うバンドエイド型ペプチドを用いた低分子医薬品の開発

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■プロジェクト概要

癌の増殖、浸潤、転移には多くのプロテアーゼが関与している。特に、ウロキナーゼは癌細胞に発現しているウロキナーゼ受容体に結合することで増殖、浸潤が開始される。今までウロキナーゼとウロキナーゼ受容体の結合を阻害する多くの阻害薬が開発され、基礎的臨床的に検討が行われてきた。

今回はウロキナーゼとウロキナーゼ受容体の結合をコンピュータ計算することで阻害薬の開発を行った。ウロキナーゼの20-26番目のアミノ酸がその結合には重要であることが判明した。詳細な検討により、ウロキナーゼのLys23, Lys46, Lys98とLys61が結合には重要なアミノ酸であることも分かった。そこでこれらのアミノ酸をそれぞれの組み合わせでLeuに変換し、結合に与える影響を計算した。その結果、ウロキナーゼ受容体のポケットの最深部に存在する23番目よりLys46とLys98が最も重要であることが確認できた（豊橋技術科学大学 栗田典之先生との共同研究）。これはポケットの入り口に存在するので、この結合を阻害する「バンドエイド型ペプチド」の設計、試作に取り組んだ。基本骨格はGly-Lys-Gly-Glu-Gly-Glu-Gly-Lys-Glyであり、これをH1ペプチドとし、より親和性を上げる改良を加えH2, H3, H4, H5, H6, H6,H7とH8を設計した。

卵巣がん培養細胞TOV-21GとSKOV3を用いたin vitro実験で、H5が増殖抑制、浸潤抑制効果が最も高いことがわかり、以後H5を用いたin vivo実験を行った。ヌードマウスを用いた皮下移植実験でH5単独で51%の増殖抑制効果をもたらし、シスプラチンとの併用で62%の抑制効果が確認された。またH5に対する獲得耐性は認められなかった。マウスの体重減少は5-10%程度のため大きな副作用はないと考えている。

対 象 疾 患：卵巣癌
特 許 情 報：なし
技術の特徴：薬剤耐性を回避できる
市場性、開発における課題：疾患拡大を図る
希望する企業連携の内容(共同、ライセンスアウト等)、企業とアカデミアの役割分担を明確にする情報

A novel peptide blocking cancer cell growth and invasion by structure-based drug design

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■Project Outline

Recent biochemical studies have revealed that a variety of proteases play important roles in cancer invasion and metastasis. Among these proteases, urokinase-type plasminogen activator (uPA) is particularly important, since its specific binding to the receptor (uPAR) expressing on the surface of a cancer cell is considered to be a trigger for cancer growth and invasion. To develop a potent inhibitor for the binding, many types of peptides of amino acids have been produced and their effect on the cancer growth and invasion has been investigated in the previous biochemical experiments. In the present study, we determined the structure of uPA and uPAR complex in water and investigated the specific interactions between uPA and uPAR by ab initio molecular orbital (MO) calculations based on fragment MO method.

The result indicates that the 20-26 amino acid residues of uPA are important for the binding between uPA and uPAR, and that the electrostatic interactions between the charged amino acid residues existing in both uPA and uPAR have large contribution to the binding. In particular, Lys23, Lys46, Lys98 and Lys61 of uPA are found to have strong attractive interactions with uPAR. To elucidate the effect of these residues on the interactions between uPA and uPAR, we substituted each of them with the uncharged amino acid Leu and investigated the interactions between the mutated uPA and wild-type uPAR. The interaction energies indicate that Lys46 and Lys98, which bind uPA to the rim of the central ligand-binding cavity of uPAR, make greater contributions to the binding between uPA and uPAR than Lys23, which is positioned at the bottom of the ligand-binding cavity of uPAR. We propose some peptides composed of these important residues and investigate the specific interactions and the binding affinity between uPAR and the peptides at an electronic level, using ab initio molecular simulations. We identified an initial peptide containing the Gly-Lys-Gly-Glu-Gly-Glu-Gly-Lys-Gly sequence (peptide H1), which strongly interacts with uPAR. With computational prediction algorithms and structure-based drug design, additional synthetic peptides, including H1, H2, H3, H4, H5, H6, H6, H7 and H8 have been produced. Base on the results simulated, we elucidate which peptide can bind more strongly to uPAR and propose a novel potent peptide which can inhibit the binding between uPAR and uPA efficiently.

Herein, we report the most effective peptide H5 in ovarian cancer cell lines, TOV-21G and SKOV3. H5 also synergized currently clinically used cisplatin in in vitro inhibition of TOV-21G and SKOV3 cell viability and re-sensitized inhibition of in vivo tumor growth. In vivo studies in a TOV-21G xenograft model showed that H5 (5 mg/kg, day 7, 10, 13, 16, and 19; i.p.) inhibited tumor growth by 51% compared to vehicle-treated mice. Compared to vehicle-treated mice, TOV-21G xenograft studies also showed that H5 (5 mg/kg/day, day 7, 10, 13, 16, and 19; i.p.) and cisplatin (100μg/kg, day 7, 13, and 19; i.p.) inhibited tumor growth by 62%. Cancer cells did not eventually develop acquired resistance to the uPAR inhibitor, H5. These results are expected to be informative for developing new peptide antagonists that block the binding of uPA to uPAR in the cancer patients and improving their prognosis dramatically.

Target : Ovarian cancer

Licencing : none

Technical characteristics : To prevent acquired resistance.

marketability : To expand indication.