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Production and characterization of monoclonal antibodies against Human Her2 and evaluation of its effect on breast cancer cell line SK-BR-3

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Papers included in the thesis

In this thesis, I present my work in on the following four papers:

- **Linear and Conformational B Cell Epitope Prediction of the HER 2 ECD-Subdomain III by in silico Methods**
  Manijeh Mahdavi, Hassan Mohabbatkar, Mehrnaz Keyhanfar, Abbas Jafarian Dehkordi, Mohammad Rabbani.
  Asian Pacific J Cancer Prev. 2012, 13, 3053-3059.

- **In silico design of discontinuous peptides representative of B and T-cell epitopes from HER2-ECD as potential novel cancer peptide vaccines**
  Manijeh Mahdavi, Violaine Moreau, Mehrnaz Keyhanfar, Hassan Mohabatkar, Abbas Jafarian Dehkordi, Mohammad Rabbani.
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- **Immunization with a novel chimeric peptide representing B and T-cell epitopes from HER2 extracellular domain (HER2 ECD) for breast cancer**
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- **Production and characterization of new anti-HER2 monoclonal antibodies**
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ABBREVIATIONS

Ab	antibody
APC	Antigen-presenting cell
BSA	bovine serum albumin
CTL	cytotoxic T lymphocyte
ECD	extracellular domain
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
HER	human epidermal growth factor receptor
mAb	monoclonal Ab
MAP	multiple antigenic peptide
MHC	major histocompatibility complex
MTT	C,N-diphenyl-N'-4,5-dimethyl thiazol-2-yl tetrazolium bromide
MVF	measles virus fusion protein amino acids 288-302
NK	natural killer
PBS	phosphate-buffered saline
TAA	tumor-associated antigen

Production and characterization of monoclonal antibodies against hHER2 and evaluation of its effects on breast cancer cell line SK-BR-3

Abbas Jafarian, Mehrnaz Keyhanfar, Mohammad Rabbani, Hassan Mohabatkar and Manijeh Mahdavi.

Abstract

Introduction: Worldwide, breast cancer is a major public health problem. Although, in Iran cancer is the third cause of death after coronary heart disease and accidents, it's mortality is on the rise during recent decades. About 15% to 20% of patients with invasive breast cancer have abnormally high levels of the HER2 protein. HER2 is a specialized protein found on breast cancer cells that controls cancer growth and spread.

Methods: This study describes generation and characterization of new anti-HER2 mAbs towards HER2 protein using a chimeric peptide immunogen containing discontinuous B-cell epitope peptide (peptide 626) and promiscuous T-helper epitope (MVF). The chimeric peptide was designed by bioinformatics analysis. MAbs were isotyped using isotyping kit. They characterized by SDS-PAGE, Western blotting, and immunofluorescence. The effects of purified mAbs on breast cancer (SK-BR-3) cell proliferation were evaluated by MTT assay.

Results: We generated three IgG isotype monoclonal antibodies ($1A_{11}$, $5H_5$ and $5H_{11}$) and seven IgM isotype mAbs using a standard hybridoma technology. The specificity of these mAbs was confirmed by various immunoassays, including ELISA, Western blotting, and immunofluorescence. In addition, the MTT assay results indicated that $5H_5$ and $5H_{11}$ mAbs could reduce growth of SKBR3 cells by approximately 50% ($P < 0.05$).

Discussion: These mAbs that can reduce cancer cells proliferation would be useful for cancer therapy. Furthermore, the synthetic peptide used in the current work, was able to induce immune system to generate antibodies especially IgG isotype. Therefore, it could be further used as cancer peptide vaccine that target different epitope or structural domain of HER2 ECD.

Key words: HER2 receptor, Monoclonal antibody, Antigen design, Breast cancer

تولید و تعیین خصوصیات آنتی بادیهای مونوکلونال بر ضد رسپتور Her2 انسانی و بررسی اثرات آن بر رده سلولهای سرطانی سینه SK-BR-3

عباس جعفریان دهکردی، مهرناز کیهانفر، محمد ربانی، حسن محبتکار و منیژه مهدوی

خلاصه پایان نامه

مقدمه: سرطان سینه شایعترین نوع سرطان زنان در سراسر دنیا و مهمترین دلیل مرگ آنهاست. با اینکه در ایران، سرطان بعد از بیماریهای قلبی عروقی و تصادفات عامل سوم مرگ و میر می باشد؛ این میزان در دهه های اخیر افزایش یافته است. در حدود ۱۵ تا ۲۰ درصد از بیماران مبتلا به سرطان سینه، پروتین Human Epidermal Growth Factor (HER2) Receptor 2 با میزان بالایی بیان می گردد که نقش مهمی در رشد و تکثیر سلولهای سرطانی دارد.

روشها: در این مطالعه، تولید و تعیین خصوصیات مونوکلونال آنتی بادیهای جدید بر ضد رسپتور HER2 انسانی توسط یک پپتید ایمونوژن حاوی پپتید اپی توپ B-cell ناپیوسته (Peptide 626) و اپی توپ T-cell (MVF) توضیح داده می شود. این پپتید ایمونوژن با روشهای بیوانفورماتیک طراحی گردید. اختصاصیت مونوکلونال آنتی بادیهای تولیدی با روشهای الایزا، وسترن بلات و ایمونوفلورسانس غیر مستقیم و همچنین اثرات مهاری آنها بر رشد سلولهای سرطانی SK-BR-3 با روش MTT بررسی گردید.

نتایج: در این تحقیق سه مونوکلونال آنتی بادی از نوع ایزوتیپ IgG ($1A_{11}$, $5H_5$ and $5H_{11}$) و هفت مونوکلونال آنتی بادی از نوع ایزوتیپ IgM با روش استاندارد تکنولوژی هیبریدوما تولید گردید. اختصاصیت مونوکلونال آنتی بادیهای تولیدی با آزمایشهای مختلفی از قبیل الایزا، وسترن بلات و ایمونوفلورسانس غیر مستقیم تایید گردید. علاوه بر آن تستهای MTT نشان داد که مونوکلونال آنتی بادی $5H_5$ و $5H_{11}$ تا حدود ۵۰٪ از رشد سلولهای سرطانی سینه SK-BR-3 جلوگیری می کنند ($P < 0.05$).

بحث: مونوکلونال آنتی بادیهای تولید شده در این تحقیق با توانایی کاهش در رشد سلولهای سرطانی سینه میتوانند در درمان سرطان مورد استفاده قرار گیرند. همچنین پپتید سنتتیک مورد استفاده در این تحقیق برای تولید مونوکلونال آنتی بادی قادر به تحریک سیستم ایمنی موش با تولید آنتی بادی از نوع IgG بود. بنابراین از این پپتید که به اپی توپ یا دومین ساختاری متفاوتی از قسمت خارج سلولی رسپتور HER2 اتصال می یابد؛ میتواند به عنوان پپتید واکسن سرطان در مطالعات بیشتری استفاده گردد.

کلید واژه‌ها: گیرنده فاکتور رشد اپیدرمال انسانی (HER2)، آنتی بادی مونوکلونال، الایزا، طراحی آنتی ژن، سرطان سینه

Future works

- Characterization of the ability of anti-HER2 peptide mAbs to inhibit angiogenesis, receptor interactions, tumorigenesis and ....

- Humanization of these mAbs, because they cannot use in this form for human and should be converted to human antibodies especially IgG mAbs.

- Designing new synthetic cancer peptide vaccines based on proposed strategy

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Letter of Approval

Isfahan University of Medical Sciences
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The undersigned certify that they have read and recommended to the Faculty of Pharmacy and Pharmaceutical Sciences for acceptance a thesis entitled "**Production and characterization of monoclonal antibodies against Human Her2 and evaluation of its effects on breast cancer cell line SK-BR-3**" submitted by **Manijeh Mahdavi** in partial fulfilment of the requirement for degree of **doctor of philosophy (PhD) in Pharmaceutical Biotechnology.**

This thesis is confirmed with the score ...19.35......and the grade Excellent.....

Abbas Jafarian Dehkordi.................A. J...

Mehrnaz Keyhanfar...With Best wishes M. Keyhanfar...........................

Mohammad Rabbani...

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Ali Jahanian-Najafabadi..................... with the best wishes

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on behalf

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