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Uptake of HER2 antigen by Dendritic Cells: Technical approaches in cancer immunotherapy

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Abstract:

Dendritic cells (DCs) of the immune system are the most professional antigen presenting cells (APCs), which are effective in processing and presenting of an antigen (Ag) to the T lymphocytes. The effective immune responses are relied on antigen presentation as a fundamental process for the function of the immune system. Recently, the immunotherapeutic studies have focussed on using DCs for vaccination of cancer patient based on the immunogenicity of the antigen delivered by DCs that have been isolated from the blood of that patient. Hence, DCs were used in this study to demonstrate the antigen uptake. This study is a technical work aiming to explore the uptake of breast tumour antigen (HER2) by DCs. The cryopreserved peripheral blood mononuclear cells (PBMCs) were purchased from a cell company to generate DCs. Expression of the major histocompatibility (MHC) molecules (i.e., HLA-A,B,C and HLA-DR) and cell surface receptors (i.e., CD86 and CD11c) on the cell surface of the newly generated DCs were determined. Then, DCs were examined for Ag uptake. The preliminary results demonstrated high expression of MHC molecules and the cell surface receptors and the uptake of HER2 antigen by DCs. This work may give an insight into understanding the mechanism of presentation of HER2 antigen to helper (CD4+) T cells. This kind of research would aid in designing and engineering of cancer vaccines and should be validated *in vivo* using animal model.

Keywords: Dendritic cells ; cancer vaccines ; HER2 antigen, immunotherapy; breast cancer

تعزيز إلتهام مستضد سرطان الثدي: طرق تقنية في العلاج المناعي

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الملخص:

الخلايا الغصنية هي إحدى خلايا الجهاز المناعي وهي الأكثر تخصصاً في عرض المستضدات، فهي فعالة في تكسير وعرض المستضد للخلايا الليمفاوية التائية. تعتمد الإستجابات المناعية الفعالة على عرض المستضدات كعملية أساسية لوظيفة جهاز المناعة. ركزت دراسات العلاج المناعي حديثاً على استخدام الخلايا الغصنية كلقاح لمرض السرطان بناءً على المناعة المتكونة ضد المستضد الذي تلتهمه الخلايا الغصنية المعزولة من دم المريض. ولهذا السبب تم استخدام الخلايا الغصنية في هذه الدراسة لمعرفة عملية إلتهام مستضد سرطان الثدي (هير 2) بواسطة هذه الخلايا. تعد هذه الدراسة عملاً تقنياً يهدف إلى استكشاف إلتهام مستضد سرطان الثدي بواسطة الخلايا الغصنية. تم شراء الخلايا وحيدة النواة المحيطية المجمدة من شركة للخلايا و تم استخدامها لعزل الخلايا الغصنية منها. تم تحديد وجود مركبات التوافق النسيجي النوع الأول والثاني ووجود المستقبلات الخلوية على سطح الخلايا الغصنية المعزولة، و تم إجراء اختبار الخلايا الغصنية المعزولة حديثاً لإلتهام المستضد. أظهرت النتائج الأولية تعبيراً عالي لمركبات التوافق النسيجي والمستقبلات الخلوية وإلتهام الخلايا للمستضد. ويساعد هذا العمل على فهم آلية عرض مستضد سرطان الثدي (هير 2) للخلايا التائية المساعدة. هذا النوع من البحوث مهم في تصميم وتطوير لقاحات السرطان، ويجب التحقق منه في الجسم الحي باستخدام نموذج حيواني.

الكلمات المفتاحية: الخلايا الغصنية؛ اللقاحات السرطانية؛ هير 2؛ العلاج المناعي؛

سرطان الثدي

Introduction:

DCs are the most effective APCs in processing and presenting of an antigen to T lymphocytes. Peptide epitopes are generated upon processing of an antigen which is bound to MHC class I or class II molecules for presentation to CD8⁺ or CD4⁺ T cells, respectively, on the surface of APC. Uptake and internalization of an antigen (exogenous antigen) is the first and basic step of antigen presentation pathway (Bryant, et al., 2002; Hiltbold & Roche, 2002). Therefore, the mechanism of Ag uptake is a fundamental process for effective antigen processing and presentation. From the previous studies, it was found that DCs have a unique capacity to activate and regulate adaptive immune response (Alhoderi & Fernandez, 2022). One of such studies used dendritic cells transfected with messenger RNA from tumour cells for induction of cytotoxic T cell responses and tumour immunity (Boczkowski, et al., 2000). This is a type of dendritic cell-based vaccines using an RNA of a tumour antigen. The recent immunotherapeutic studies have focussed on using DCs as adjuvant for vaccination (Banchereau & Palucka, 2005; Mkrtichyan, et al., 2011), hence the immunogenicity against the antigen delivered by DCs have been reported in cancer patients (Banchereau & Palucka, 2005). The aim of those studies is to demonstrate the uptake of tumour antigens by APCs and how to direct those antigens to the antigen processing compartments for presentation effectively to T lymphocytes (Alhoderi & Fernandez, 2022). This approach could facilitate the development of effective tumour immunotherapies and/or tumour vaccines.

Human epidermal growth factor receptor 2 (HER2) is a transmembrane glycoprotein receptor of the epidermal growth factor receptor family and highly expressed in several solid tumours, particularly in breast cancer (Budi, et al., 2022; Cheng, 2024; Robbins, et al., 2025). Breast cancer is the most prevalent type of cancers in the world and considered the second cause of death in women due to tumour heterogeneity, drug resistance, and adverse side effects (Gao, et al., 2024). HER2 overexpression is associated with tumorigenesis and metastasis. (Zhu, L. et al., 2025). Recent reviews provide an overview of HER2 biology, as well as therapeutic strategies targeting HER2 in cancer, because deep

understanding of HER2 regulation and the accuracy of its testing is the basis for treatment strategies and developing effective therapies (Budi, et al., 2022; Cheng, 2024; Robbins, et al., 2025). Accurate identification of biomarkers for tumours is the key for diagnosis and treatment of cancer. Emphasis was given to HER2 (Gao, et al., 2024; Turk, et al., 2024). Hence, HER2 has been a fundamental element for selection of patients who are at low levels of HER2 expression for therapy (Robbins, et al., 2025). HER2/neu I is another oncogenic cell-surface protein that induces cellular and humoral immune responses. It expresses on human breast cancer cells and detected by antibodies and recognized by T cells. HER2/neu antigen is homologous with epidermal growth factor receptor and recent research focused on it to conduct of clinical trials (Berzofsky, et al., 2019., Costa, et al., 2019., Pallerla, et al., 2021., Corti, et al., 2022). In the present study, DCs were used as APCs. The main aim of this work was to look at the uptake of breast tumour antigen (HER2) by the newly generated DCs, as a model of DC-based tumour vaccine. This technique will highlight two characteristics related to antigen presentation. One is the property of antigen uptake by DCs. The other is the capacity of DCs in delivering the antigen to the intracellular compartment and enhancing the immune response.

Materials and Methods:

Separation and culture of monocytes

The cryopreserved human PBMCs (Cat no: CTL-UP1; CTL Company) were purchased from the CTL company as small aliquots (about 1.6ml each), each aliquot has more than million cells with over 96% viability and 100% functionality. They were frozen under serum-free conditions. The ethical approval has been obtained from the school of life sciences at university of Essex. The procedure of culture the cryopreserved human PBMC is provided by the supplier. The vial of PBMC was thawed using one vial of CTL-Anti-Aggregate-washTM Supplement 20x (Cat no: CTL-AA-001; CTL Company). The cells were cultured at 5×10^6 cells in a big flask and incubated for 1 hr at 37° C to be separated into adherent and non-adherent cells.

Generation of dendritic cells

After the incubation time, the non-adherent cells were collated and frozen for future use, and the adherent cells were stimulated with

GM-CSF (Cat no: 130-093-862; Miltenyi Biotec) and IL-4 (Cat no: 130-093-915; Miltenyi Biotec), at 1000 IU/ml of each for 5 days. Afterwards, they were stimulated with TNF- α (Cat no: 130-094-015; Miltenyi Biotec) at 1000 IU/ml for another 2 days to mature the cells.

Harvesting the dendritic cells

DCs were harvested by using cold Hank's buffer (HBSS, Cat. no. H15-008; PAA). The flask of DCs was kept for a little time on the ice, on the surface where the DCs are growing. After that, the flask was shaken vigorously and washed several times with sterile Hank's buffer. After each rinse with Hank's buffer the cells were checked under microscope to see whether they were detached. Then, they were centrifuge for 1000 rcf for 5 mins and adjusted at the recommended cell number for each experiment.

Phenotype the dendritic cells

DCs were characterized in terms of expression of the receptors on the cell surface of the cells. Indirect cell surface immunostaining followed by flowcytometric analysis was applied. Mouse anti-human monoclonal antibodies were used for HLA-A, B, C at 25 μ g/ml (Cat no: 311415; Cambridge Biosciences); HLA-DR at 25 μ g/ml (Cat no: AB4869; R & D Systems); CD86 at 20 μ g/ml (Cat no: 21480861; Immuno Tools) and CD11c at 20 μ g/ml (Cat no: 21487111; Immuno Tools) . Cells only, cells with secondary antibody (Rabbit anti-mouse FITC: RAM-FITC; Cat no. F9137; Sigma), and cells stained with anti-CD14 antibody (Supernatant; Cambridge) were used also as negative controls. No statistical analysis was applied as the results compared with the negative control and the experiment was repeated twice.

Antigen uptake assay

Antigen uptake assay was carried out as one of the main characteristics of the professional antigen presenting cells. Breast tumour antigen HER2 conjugated with FITC (Cat no: HE2HF224-25 μ g; ACROBiosystems) was used to test the ability of the DCs for Ag uptake. DCs (300,000 cells/well) were cultured in a chamber slide and stimulated with 1 μ l of IFN- γ (Cat. no: 130-096-872; Miltenyi Biotec) at 10 ng/ml overnight at 37° C in the incubator. On the following day, they were pulsed with FITC-HER2 (25 μ l at 25 μ g/ml) and incubated for 30 min at 37° C in the incubator. Cells were washed with PBS three times, fixed and washed again. Then they

were mounted and looked under the microscope. Cells only, cells with secondary antibody (rabbit anti mouse conjugated with fluorescein isothiocyanate; RAMFITC), and cells with anti-CD86 antibody were used as controls. Another set of the samples of Ag uptake assay were treated with 0.1% sodium azide; NaN₃ (Cat no: S2002, Sigma- Aldrich) before pulsing the DCs with an Ag to prevent the internalization of an antigen and confirm the ability of DCs for HER2 uptake (i.e., a negative control assay). The experiment was repeated twice. Image analysis was performed after image capture and one-way ANOVA was applied for statistical analysis of the data. A schematic diagram summarizing the methods and techniques applied in this study is depicted in Figure 1.

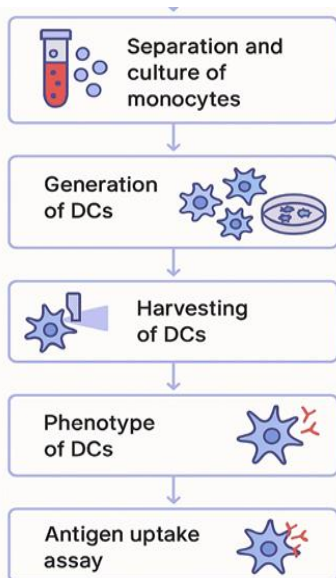


Figure 1. The workflow of the methods and techniques

Results:

Phenotyping of the receptors

Flow cytometry technique was applied to detect the expression of CD86 and CD11c on the cell surface of the DCs. Figure 2 shows the expression of CD86 (right panel; 86%) and CD11c (left panel; 93.3%) on the surface of DC. Another set of experiment (Figure 3) was done to detect the expression of two other receptors those express on all types of APC (i.e., MHC class I and MHC class II).

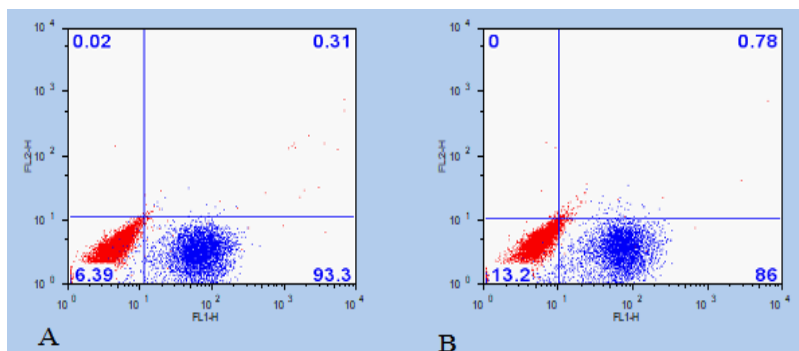


Figure 2. Expression of the receptors on the cell surface of DCs. Isotype (red colour) were used as a negative control. The results showed a good expression of CD11c (A), CD86 (B); (blue colour)

Immunofluorescence (IF) staining experiments were also carried out to phenotype the DCs by detection of their specific receptors (CD86 and CD11c) using monoclonal antibodies specific for those receptors. Then, the labelled cells in a slide were looked under the microscope. That was done to confirm the results of flow cytometry. Figure 4 shows the cells expressed these receptors under the BX41 (60x magnification). Figure 5 demonstrate the images of HLAA,B,C and HLA-DR molecules on the surface of DCs.

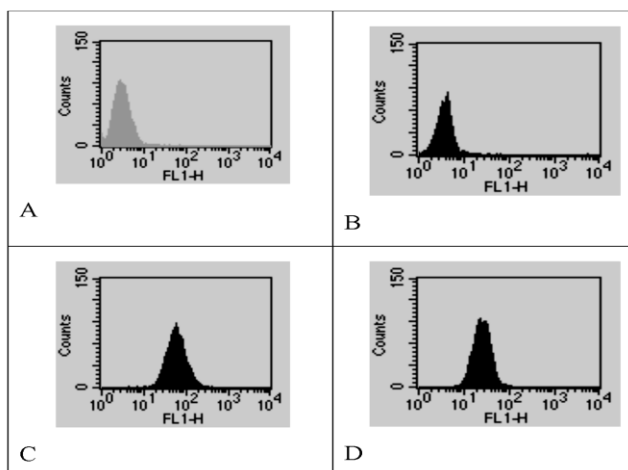


Figure 3. Expression of MHC class I (HLA-A, B, C), and MHC class II (HLA-DR) on the surface of DCs. The results showed positive expression of HLA-DR (C) & HLA-A,B,C (D). Cells stained with isotype (B) and cells only (A) were used as negative controls.

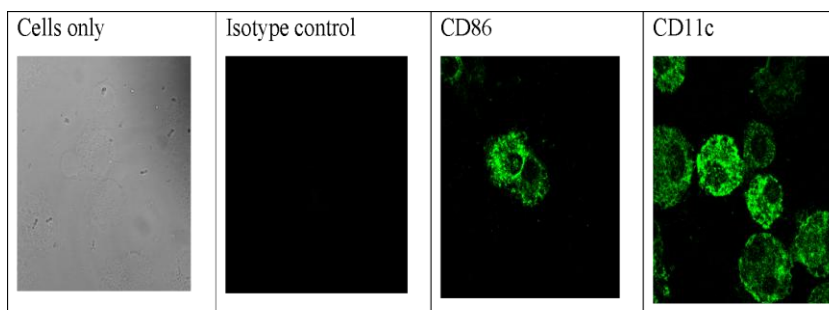


Figure 4. Expression of the receptors on the cell surface of DCs using IF staining. The result showed a surface expression of CD86 and CD11c. Cells only and cells with isotype were used as negative controls.

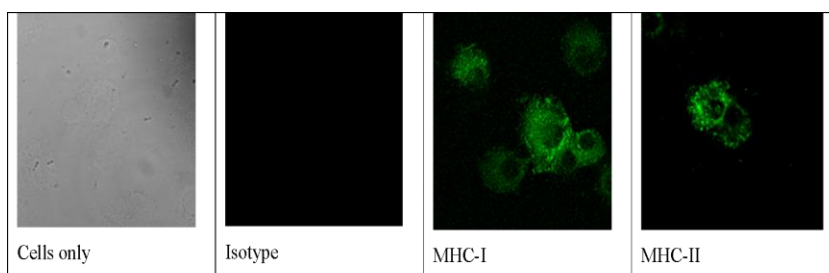


Figure 5. Expression of MHC class I (HLA-A, B, C) and MHC class II (HLA-DR) using IF. The images show the expression of both types of MHC molecules on the cell surface of DCs. Cells stained with isotype and cells only were used as negative controls. BX41 microscope was used for imaging (60X magnification).

Antigen uptake

Ag uptake assay was carried out by using FITC-HER2 as a breast tumour Ag to test the ability of the newly generated DCs for antigen uptake. The results demonstrated the uptake of FITC-HER2 by dendritic cells. That gives insights into the professional function of the generated dendritic cells for uptake of the antigen. Figure 6 presents the result of this assay. A negative control assay (Figure 7) for Ag uptake was adopted by treating another set of the samples with 0.1% of Sodium Azide (NaN₃). The results showed that HER2 uptake by the treated cells was blocked and DCs failed to uptake the Ag. The control assay confirmed the uptake of HER2 by DCs in the experimental assay. One-way ANOVA was applied for statistical analysis of the data (p-value < 0.05 ***).

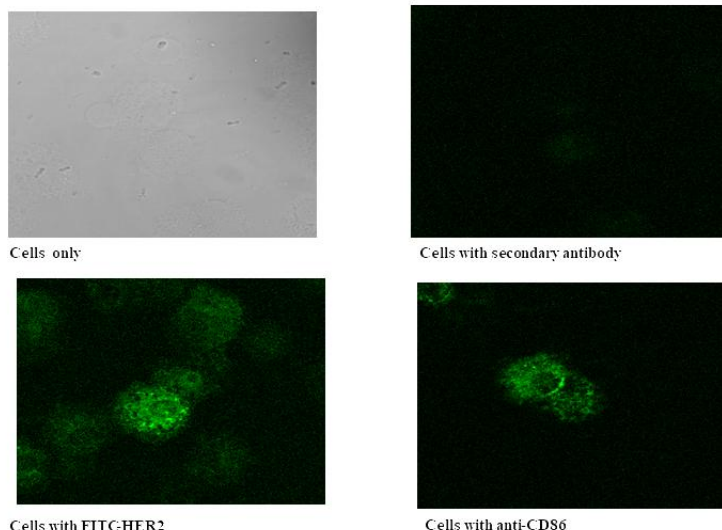


Figure 6. Antigen uptake assay. DCs were cultured and pulsed with FITC-HER2. Images were captured by the microscope (BX41 at 60X magnification). Cells only, cells with secondary Ab and cells with anti-CD86 Ab were used as controls. The results demonstrated the uptake of an antigen by DCs.

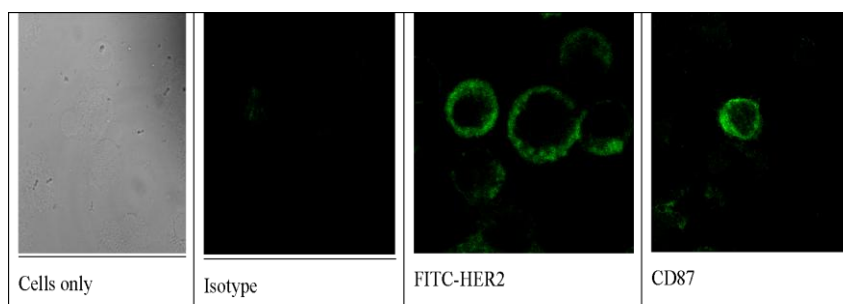


Figure 7. DCs were treated with 0.1% NaN₃ (a negative control assay). The results showed that HER2 uptake was blocked and DCs failed to uptake the Ag.

Discussion:

The presented work aimed to demonstrate the uptake of a breast tumour Ag (HER-2) by DCs. HER2 is one of the cancer testes (CT) antigens which has been shown as an immunogenic tumour Ag (Berzofsky, et al., 2019; Costa, et al., 2019; Pallerla, et al., 2021). Previous studies have determined several T-cell epitopes of HER2

that elicited cytotoxic (CD8⁺) T cell and helper (CD4⁺) T cells responses against cancer cells express HER2. That may have impacts on the design of peptide-based cancer vaccine (Kobayashi, et al., 2000; Sotiriadou, et al., 2001; Baxeavanis, et al., 2004; Tobias et al., 2022). In the current work, DCs were generated from PBMCs of blood donors and used for the study. The isolation of monocyte from PBMCs has made the generation of monocyte-derived dendritic cells (Mo-DCs) more efficient and can be applied in biological and clinical approaches. DCs are the most effective professional cells for uptake, processing and presentation of an antigen. Several studies have used DCs for studying tumour immune responses (Gall, et al., 2017; Basu, et al., 2019; Berzofsky et al., 2019). The expression of the specific receptors on the newly generated DCs was assessed to confirm the successful generation of DCs. The Ag uptake assay in this study provides an indication of the quality and ability of the newly generated DCs in uptake of HER2. Previous study has shown that the vaccination of breast cancer patients with HER2-pulsed DCs has reduced the expression of HER2 in breast tumour cells (Basu, et al., 2019). Similar optimistic results have shown that using autologous DCs pulsed with HER2 had successful clinical outcomes in 54% of patients (Berzofsky, et al., 2019). Furthermore, it has been demonstrated that targeting HER2 with the monoclonal antibody (trastuzumab) increases the uptake of HER2 (Gall, et al., 2017). Several studies in tumour immunology have focussed on the identification of tumour antigens that develop antigen-specific T-cell responses (Alhoderi & Fernandez, 2022) and using of DCs as a moder of cell-based cancer vaccine (Baldin, et al., 2020; Pallerla, et al., 2021). Recent reviews have highlighted the current clinical trials of anticancer DC vaccination and the latest investigations about DC vaccines (Najafi & Mortezaee, 2023; Zhu, T. et al., 2025). Therefore, focusing on uploading of DCs by the immunogenic tumour antigens could have a potential application of DC vaccines. This kind of research would aid in designing of peptide-based cancer vaccines as in the most recent studies (Firuzpour, et al., 2025; Soliman, et al., 2025)

Conclusion:

DCs were generated from the purchased cryopreserved human PBMCs and examined for expression of the receptors. High

expression of MHC molecules and receptors on the cell surface of DCs were determined. Ag uptake by DCs was demonstrated as well. The preliminary results showed the ability of DCs in uptake of HER2 antigen. These results may provide an insight into understanding of the presentation of tumour antigens to helper T cells. In conclusion, DCs have been a significant target for research in developing cancer vaccines, and more studies should be conducted *in vivo* using experimental animals.

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