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授与した学位	博　士
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学位論文の題目	Study on the biophysical property of recombinant cancer-testis antigens for autoantibody biomarker analysis （自己抗体バイオマーカー解析のための組換えがん精巣抗原の生物物理学的特性に関する研究）
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学位論文内容の要旨

Study on the biophysical property of recombinant cancer-testis antigens for autoantibody biomarker analysis

At present, in addition to the conventional three major treatments for cancer, surgery, radiotherapy, and chemotherapy, immunotherapy is attracting attention as a fourth cancer therapy. Immunotherapy can mobilize the immune system against cancer has led to remarkable effects on some advanced cancers that cannot be considered by conventional treatments. However, the success is limited to a subset of patients that have immunity hot-tumor in which immune checkpoint inhibitors work well, and the remaining with immunity cold-tumor do not respond to immunotherapy. This heterogeneous response is closely related to the individual variety of immunogenicity of cancer cells and antitumor immune response. To realize precision medicine on cancer-immune therapy, monitoring the tumor microenvironmental immune response using a small aliquot of peripheral blood will be a great tool for clinical decisions. Because the activation of the cancer-immunity cycle is known to essential for good clinical outcomes, the biomarker reflecting this activity is an important target. Our laboratory focusing on autoantibody because immunologically rejected cancer cells contain cancer-specific antigens activating anti-cancer immunity. Because the cancer-specific antigens vary in individuals, the development of multiplexed autoantibody biomarker quantitative detection system is important. The changing the autoantibody levels reflecting the level of the cancer immunity cycle. To develop this assay system, it is necessary to understand the biophysical properties of cancer-specific antigens. Although cancer-specific antigens are varied in individuals, we focused on cancer-testis antigens (CTAs), which are immunogenic proteins that are normally expressed in testis and often aberrantly expressed in cancer cells. A recent clinical study revealed that serum levels of anti-CTA antibodies are closely related to activated anti-tumor immune responses, as well as these antibody levels could be a critical biomarker for improved cancer immunotherapy. My research was focused on following two respects for anti-CTA antibody detection study.

1. Analysis of unusual aggregation property of recombinantly expressed cancer-testis antigens in mammalian cells

Transient expression of human intracellular proteins in the human embryonic kidney (HEK) 293 cells is a reliable system for obtaining soluble proteins with biologically active conformations. Contrary to conventional concepts, we found that recombinantly expressed intracellular cancer-testis antigens (CTAs) showed frequent aggregation in HEK293 cells. Although experimental subcellular localization of recombinant CTAs displayed proper cytosolic or nuclear localization, some proteins showed aggregated particles in the cell. This aggregative property was not observed in recombinant housekeeping proteins. No significant correlation was found between the aggregative and biophysical properties, such as hydrophobicity, contents of intrinsically disordered regions, and expression levels, of CTAs. These results can be explained in terms of the structural instability of CTAs, which are specifically expressed in the testis and aberrantly expressed in cancer cells and function as a hub in the protein-protein network using intrinsically disordered regions. Hence, we speculate that recombinantly expressed CTAs failed to form this protein complex. Thus, unfolded CTAs formed aggregated particles in the cell.

2. Preparation of mouse CTAs array for detection of autoantibody biomarker.

Evaluation of anti-tumor immune response using anti-CTAs autoantibody using the mice model has enormous potential for the immune-modulating drug discovery. To establish this system, a mouse-specific autoantibody detection system is required for the analysis of immune monitoring in the tumor-bearing immune-competent mouse. Because the protein sequence homology between human and mouse cancer antigens is quite low, it was necessary to redesign the mouse-model specific autoantigen array. Eighteen full-length mouse CTAs from their human counterpart and three TGC proteins that were reported as candidates for TGC-specific auto immunogenic antigens were expressed and purified in bacterial cells. These mouse testis-specific proteins were expressed and purified in bacterial cells. The protein expressed as insoluble inclusion bodies were solubilized by using the S-cationization technique, and soluble proteins were purified by using nickel column chromatography. On the analysis of antibody response in the combination immunotherapy received tumor-bearing mouse, antibody responses were successfully monitored by using the Luminex system. Because antibody responses were observed before tumor regression, antibody monitoring has the potential to predict the clinical outcome of treatments.

論文審査結果の要旨

本論文は，がん細胞の抗原性を担うタンパク質群で腫瘍免疫学的に極めて重要なcancer-testis（CT）抗原が，不安定で凝集しやすい物性であることを実験科学的に証明するとともに，CT抗原に対する自己抗体バイオマーカーの変動が，がん免疫治療の予測に活用できる可能性を示している。

第1章では，ヒト細胞内で発現させたヒトおよびマウスの組換え体CT抗原タンパク質が高頻度に細胞内で凝集することを70種類のヒトCT抗原と，15種類のマウスCT抗原をモデルとして網羅的に解析・証明している。免疫細胞染色では大半のCT抗原が細胞内で凝集塊を形成することも観察された。一部のCT抗原については，がん細胞株の細胞内でも凝集していることも確認し，多くのCT抗原が凝集性の高い物性であることを示した。これらの観察結果を総括し，がん細胞内で発現するCT抗原は凝集性を示す例が多く，それ故に，自己抗体が誘導されやすいと考察している。

第2章では，マウスモデルでがん免疫治療が奏功する際に，各種のCT抗原に対する自己抗体が変動することを示している。ヒトとマウスではCT抗原の配列の保存性が低いため，マウスCT抗原を中心とした抗原アレイの準備が必要なことが示された。モデル系として実施された系では免疫治療が奏功する群において，自己抗体の上昇が腫瘍縮小の前に観察されることを確認し，がん免疫治療のバイオマーカーとして利用できることを示した。

今後の研究開発が進めば，医療現場でCT抗原に対する自己抗体バイオマーカーの変動解析が治療方針の決定等に活用される可能性が高い。この際，本論文の成果は，自己抗原の物性側から考察を進める学術的基盤となる。以上のことから，審査委員全員が本論文を岡山大学大学院ヘルスシステム統合科学研究科の博士（統合科学）の学位に値すると評価した。