論文審査の要旨及び担当者

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（論文審査の要旨）

論文題名：HSP90 promotes tumor associated macrophage differentiation during triple-negative breast cancer progression
（HSP90はトリプルネガティブ乳がんの進行過程において腫瘍関連マクロファージの分化を促進する）

Tumor-associated macrophages (TAMs) derived from monocytes are critical to cancer progression; however, the mechanism of TAM differentiation is unknown. This study showed the role of HSP90 in TAM differentiation, implying that HSP90 is a potential target for triple-negative breast cancer (TNBC) immunotherapy given its regulatory role in monocyte-to-TAM differentiation.

During the dissertation defense, the committee asked why only TNBC can induce differentiation but not other cell types. The candidate explained that, based on the ELISA results, TNBC cells secreted the highest levels of HSP90 into the culture medium, and this high HSP90 concentration promoted TAM differentiation. Since the molecular mechanisms of how TNBC cells release HSP90 have not been extensively studied in previous studies, it is worth investigating in the future. The committee inquired about the differences in Western Blot results between 10 nM and 20 nM siRNA-derived culture medium-induced activation, and it was noted that 10 nM did not inhibit any pathways. The candidate explained that 10nM did not completely inhibit HSP90, which is why 20nM was used. These findings suggested that a lack of HSP90 influenced TAM differentiation. The committee inquired about the origin of CD4+ T cells as well as TAM results in the first *in vivo* experiment. The candidate replied that all *in vivo* cells were derived from tumor tissues. Because of the notable volume difference between the two groups and the absence of TAM expression changes in the long-term experiment, a short-term experiment was also carried out. In the short-term experiment, the reduction in TAM caused by the HSP90 inhibitor was validated. The committee recommended that tumor tissue be examined prior to 7 days. The candidate stated that the tumor did not grow before 7 days, so it is difficult to assess the impact of the HSP90 inhibitor prior to immune system activation.

The candidate was then asked about the definition of TAM. The candidate replied that TAM is similar to M2 macrophages. When macrophages enter the tumor microenvironment, they polarize as TAM rather than M1 or M2. TAM is primarily present in tumor tissues. The committee questioned the choice of the HSP90 inhibitor and its role in tumor therapy. The candidate stated that the mechanism between the inhibitor and TAM was limited, but the mechanism between the inhibitor and tumor cells had been demonstrated. And HSP90 was not the only factor influencing tumor growth. This study demonstrated the importance of HSP90 in TAM differentiation; however, previous research has shown that other factors, such as lactic acid, promote TAM differentiation and tumor growth. The candidate proposed that combination therapies could become a viable tumor treatment strategy in the future. The committee noted that, in addition to the pathways seen *in vitro*, other mechanisms may influence the results *in vivo*. The candidate acknowledged that the analysis of the *in vivo* experiment was insufficient. To better understand the effects of HSP90 inhibitors on TAM differentiation in TNBC, more research is needed. The committee also asked if the Western blot results were quantified. The candidate responded that the western blot was used to confirm the pathway's activation and phosphorylation, but no quantification was performed.

Finally, the committee inquired about the transport of HSP90 across the cell membrane. The candidate stated that HSP90 crossed the cell membranes via the ribosome. However, previous research corrected that HSP90 crosses cell membranes via exosomes or the CD91 receptor rather than ribosomes. Several papers also reported that HSP90 binds to polypeptides across membranes. The candidate was asked about the effect of the HSP90 inhibitor on other cancers, as well as its selectivity for cancer cells. In response, the candidate demonstrated that the HSP90 inhibitor has been shown to kill cancer cells by inhibiting ATP transport by binding to HSP90's ATP binding site in several cancer types. The candidate explained that its selectivity for cancer cells over healthy cells stemmed from the fact that tumor cells synthesized more ATP and energy than other cells. And, in cancer cells, rapid proliferation and reduced control over protein synthesis result in increased Hsp90 expression. The majority of mutated oncoproteins expressed in cancer cells that promote uncontrolled growth and proliferation are also client proteins of HSP90. These could explain why the Hsp90 inhibitor preferentially selects cancer cells over healthy cells.

In light of the foregoing, this study demonstrated the therapeutic potential of immunotherapy for triple-negative breast cancer. Based on the presented findings, the current study was approved as a valuable contribution.