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研究課題名（和文）Immune evasion of AML cells from FceRI+ cells through MEIS1 activity

研究課題名（英文）Immune evasion of AML cells from FceRI+ cells through MEIS1 activity

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研究成果の概要（和文）：急性骨髄性白血病（AML）では、転写因子MEIS1は、HOXA9で形質転換された白血病細胞のinvivoでの浸潤と増殖に不可欠です。 このプロジェクトは、MEIS1の新しい機能を明らかにしました。 MEIS1は、おそらく好塩基球の活性をダウンモジュレートすることにより、HOXA9で形質転換された白血病細胞に免疫回避能力を付与します。

研究成果の学術的意義や社会的意義
This project helps understanding the mechanisms underlying the escape of leukemic cells from the immune defense system. It may lead to the development of either leukemia immunotherapeutic strategies or curative treatment aimed at annihilating the immune escape ability of leukemic cells.

研究成果の概要（英文）：Overexpression of the transcription factor HOXA9 is sufficient to immortalize hematopoietic stem cells in vitro, but co-overexpression of the transcription factor MEIS1 is necessary for in vivo invasion and propagation of HOXA9-transformed leukemic cells. We previously showed that MEIS1 overexpression is critical for bone marrow engraftment of leukemic cells following in vivo injection. However, we unraveled that the sole engraftment capacity is not sufficient for leukemia onset to occur. Indeed, we found that leukemic cells are under immune attack in vivo but have the ability to escape this immune assault.
We therefore hypothesized that MEIS1 is critical for immune evasion. This project led to the discovery that: (1) HOXA9-transformed cells are detected by FcεRI+ cells (basophils and/or mast cells) followed by eradication by T lymphocytes. (2) MEIS1 overexpression confers immune evasion capability to HOXA9-transformed leukemic cells by making cells insensitive to FcεRI+ cells.

研究分野：Leukemogenesis

キーワード：Acute Myeloid Leukemia　Meis1　Immune evasion　Oncology

１．研究開始当初の背景

Acute Myeloid Leukemia (AML) is the most frequent type of leukemia and accounts for ~90% of all acute leukemia in adults. The five-year survival rate of patients with AML is the poorest of all leukemia type with only 40% survival, which highlights the urgent necessity to improve therapy, and thereby implying a better comprehension of the molecular mechanisms governing the onset and progression of the disease.

In 50-60% of AML patients, the transcription factors HOXA9 and MEIS1 are found to be upregulated. Interestingly, co-expression of HOXA9 and MEIS1 is sufficient to transform primary bone marrow stem cells into myeloid leukemic cells able to induce AML, demonstrating the critical role of these two transcription factors in AML.

HOXA9 is a DNA-binding homeobox protein and MEIS1 is a TALE-class homeodomain protein that acts either as a DNA-binding cofactor of HOXA9 or an independent transcription factor. Within the hematopoietic system, HOXA9 and MEIS1 are normally expressed in stem cells and immature progenitor compartments, but they are downregulated during myeloid differentiation. **In AML, downregulation of HOXA9 and MEIS1 does not occur**. This leads to a differentiation block and the subsequent expansion of immature myeloid progenitors unable to generate mature effector cells, thereby leading to AML. Aberrant overexpression of HOXA9 and MEIS1 in hematopoietic stem cells is therefore an adverse event with dramatic health consequences.

HOXA9 and MEIS1 have distinctive functions within myeloid leukemic cells. Indeed, while overexpression of HOXA9 is sufficient to induce transformation and immortalization of bone marrow cells in vitro, MEIS1-overexpression cannot induce this process.
Interestingly, HOXA9-overexpressing immortalized cells are however unable to induce AML when inoculated in vivo. For in vivo propagation, co-expression of both HOXA9 and MEIS1 is critically required, demonstrating that essential target genes and functions exclusively regulated by MEIS1 are required for leukemia burden. This observation also suggests that the in vivo environment is naturally non-adequate for HOXA9-transformed leukemic cells and that the mere immortalization of hematopoietic stem cells does not confer the ability to induce leukemia.
Therefore, MEIS1 is the key factor that provides in vivo survival adaptation to HOXA9-transformed cells, and produces the necessary element(s) for long-term expansion of leukemic cells. **However, the precise function of MEIS1 and the identity of its target genes critical for AML propagation have remained elusive so far.**

２．研究の目的

We hypothesized that MEIS1 could confer immune evasion capacity to leukemic cells, **a concept that has never been investigated**. Indeed, it is well known that leukemic cells are under immune attack in vivo but have the ability to escape this immune assault. However, the exact way AML cells are detected by the immune system and the precise nature of the induced immune response are still unclear. In addition, the mechanisms permitting leukemic cells to escape from this immune attack are still elusive. We therefore proposed that one of the key in vivo function of MEIS1 is to settle the immune evasion capacity of leukemic cells by producing target genes critical for this process.
The aim of this project was therefore to identify the nature of the immune response generated by leukemic cells inoculation and, most importantly, to unravel the immune evasion mechanisms employed by AML cells to escape this immune attack, focusing onto the role of MEIS1 in this process. Indeed, we hypothesized that MEIS1 might be a master regulator for the immune evasion of leukemic cells by producing specific target genes necessary for immune escape.

In this regard, we attempted to answer several questions:
(1) Are acute myeloid leukemic cells under immune attack in vivo?
(2) Is MEIS1 responsible for the immune evasion of leukemic cells?
(3) What is the identity of the immune cells responsible for the attack?
(4) How do immune cells interfere with leukemic cells?
(5) What are the critical MEIS1-target genes responsible for immune evasion?

３．研究の方法

Hematopoietic stem cells from B6-Rosa26-Cre-ERT2 mouse bone marrow were in vitro isolated and retrovirally spinfected with Hoxa9 and Meis1 genes (cloned in pMYs retroviral vectors: pMYs-Hoxa9-IRES-mKO and pMYs-**loxP**-Meis1-IRES-EGFP-**loxP**), resulting in stably HOXA9/MEIS1-overexpressing cells (H9M1

cells) sorted on mKO/EGFP double-positive status until >95% purity.

For Meis1 transgene deletion, tamoxifen was added to H9M1 cells leading to loxP recombination and removal of Meis1 transgene, resulting in HOXA9-DeltaMeis1 cells (HΔM cells) sorted on mKO positive status until 100% purity.

Syk (Spleen Tyrosine Kinase) is a target gene of MEIS1 and has been shown to promote leukemogenesis of HOXA9/MEIS1-overexpressing cells (Mohr et al., Cancer Cells (2017), Vol. 31(4), p.549-562). Therefore, HΔM cells were retrovirally spinfected with the Syk gene (pMYs-Syk-IRES-EGFP), resulting in stably HOXA9-DeltaMeis1-SYK-overexpressing cells (HΔM-SYK cells) sorted on mKO/EGFP double-positive status until >95% purity.

All three cell lines (H9M1, HΔM and HΔM-SYK cells) therefore originated from the same clone.
For ruling out any artifactual outcome due to uncontrolled gene insertion, a second set of cell lines was created (H9M1*, HΔM* and HΔM-SYK* cells) for validation of the initial results.

For in vivo experiments, cells were inoculated intravenously into non-irradiated mice and leukemia onset was investigated through blood smear examination and flow cytometry analysis of white blood cells. Noteworthy, the avoidance of irradiation kept intact the animal immune system.

In vitro, splenic cells were co-cultured with leukemic cells during 10 days in the presence of Interleukin-3. A fraction of the co-culture was collected every day and analyzed by flow cytometry to determine the percentage of leukemic cells, as an indicator of leukemic cells expansion.

４．研究成果

(1) Are myeloid leukemic cells under immune attack in vivo?
H9M1 cells were in vivo inoculated into non-irradiated immune competent (C57Bl/6 mice) and immune deficient background (NOD/SCID and/or $RAG2^{-/-}$ mice). As expected, leukemia onset occurred more rapidly in immune deficient backgrounds.
This result demonstrated that H9M1 cells are under immune attack in C57Bl/6 mice, but have the ability to escape this immune assault.
Noteworthy, when H9M1 cells were inoculated into allogeneic BALB/c mice, leukemia did not occur due to the complete eradication of cells, demonstrating that H9M1 cells are killable when recognized by the immune system.

(2) Is MEIS1 responsible for the immune evasion of leukemic cells?
HΔM and HΔM-SYK cells were in vivo inoculated into immune competent (C57Bl/6 mice) and immune deficient background (NOD/SCID and/or $RAG2^{-/-}$ mice).
HΔM cells were unable to induce leukemia in any of the backgrounds, while HΔM-SYK cells could expand in immune deficient mice. **This result demonstrated that HΔM-SYK cells have engraftment capability but that the immune system is responsible for their eradication in C57Bl/6 mice.**

Thus, unlike H9M1 cells, HΔM-SYK cells are immunologically perceptible and their presence triggers an immune response that is fully detrimental for their survival. **In other words, SYK-overexpression is sufficient to bypass MEIS1 for bone marrow engraftment, but not for immune evasion.**
Therefore, in addition of providing the ability to interact with the bone marrow environment, we uncovered a supplementary function for MEIS1, which is to confer the ability for leukemic cells to escape the immune system attack. **Consequently, MEIS1 is responsible for the immune evasion of leukemic cells (through a Syk-independent mechanism).**

(3) What is the identity of the immune cells responsible for the attack?
In order to clarify the precise nature of this immune response, mice deficient in different compartments of the immune system were inoculated with HΔM-SYK cells and their expansion was tracked weekly.

As mentioned previously, HΔM-SYK cells could expand in $RAG2^{-/-}$ mice, suggesting the involvement of the lymphocyte compartment (T and B cells) in their eradication. A narrower investigation was undertaken through the usage of $CD3^{-/-}$ (T cell deficient) and μMT (B cell deficient) mice. HΔM-SYK cells could induce leukemia in μMT mice but not $CD3^{-/-}$ mice, demonstrating that T cells are responsible for their eradication.
The role of Natural Killer (NK) cells was also investigated by inoculation of HΔM-SYK cells into NK cells-depleted C57Bl/6 mice through injection of a depleting antibody (anti-NK1.1 Ab). HΔM-SYK could not induce leukemia, suggesting that NK cells are not involved in this process of elimination.

Finally, involvement of basophils was verified by usage of Mcpt8-DTR mice injected with Diphtheria Toxin

for depletion. HΔM-SYK cells could induce leukemia, suggesting that basophils are involved in this process of elimination.

Overall, the survival of HΔM-SYK cells into specifically depleted immune compartments indicated that T cells (presumably cytotoxic CD8 T cells) and basophils are both responsible for their eradication in vivo.

(4) How do immune cells interfere with leukemic cells?
In parallel to in vivo experiments, in vitro co-culture of leukemic cells with splenic cells was performed using wild-type or immune deficient spleens, and proliferation of leukemic cells was time-tracked.

When co-culturing H9M1 cells with wt splenic cells, the follow up showed a biphasic mode with an initial expansion of leukemic cells up to day 5-6 (Phase 1) followed by a sharp elimination phase until day 7-8 (Phase 2). By day 10, no H9M1 cells were remaining in the co-culture.
When H9M1 cells were co-cultured with RAG2$^{-/-}$ splenocytes, Phase 2 did not occur and leukemic cell number remained stable from day 5 to 10. This result demonstrated that, in vitro, H9M1 cells are eliminated by T cells at day 5-6 and cannot escape this immune assault.

On the other hand, HΔM and HΔM-SYK cells did not expand when co-cultured with either wt or RAG2$^{-/-}$ splenocytes. Therefore, the absence of MEIS1 affects the ability of cells to expand during Phase 1.
Since this phenomenon occurs even with RAG2$^{-/-}$ splenocytes, it suggests that innate immune cells are responsible for the inhibition of HΔM and HΔM-SYK cells during Phase 1, while H9M1 cells are unaffected.
Therefore, MEIS1 protects leukemic cells from proliferation inhibition mediated by innate immune cells during Phase 1.

Depletion of FcεRI^{+} cells (basophils and mast cells) from splenocytes prior to co-culture with leukemic cells restored the ability of HΔM and HΔM-SYK cells to expand during Phase 1, at a similar level than H9M1 cells. Surprisingly, Phase 2 was also affected by FcεRI^{+} cells depletion since none of the leukemic cells were eliminated by T cells. These results suggest that the cellular and molecular events occurring during Phase 1 are necessary for Phase 2 to be achieved. In other words, basophils (and/or mast cells) are necessary for T cells to start killing leukemic cells.

Interestingly, transfer of supernatant from wt splenocytes cultured for 4 days (here named wt-S4) was sufficient to inhibit proliferation of HΔM and HΔM-SYK cells in vitro, but not H9M1 cells, suggesting that basophils inhibit HΔM and HΔM-SYK cells through secreted factor(s). The same phenomenon could be observed with supernatant collected from RAG2$^{-/-}$ splenocytes cultured for 2 days (here named RAG2-S2).

We performed LC-MS/MS proteome analysis of wt-S4 and RAG2-S2 and identified 7 common factors: transmembrane glycoprotein NMB, C-C motif chemokine 6, macrophage metalloelastase, cathepsin L1, urokinase-type plasminogen activator, prosaposin and cathepsin D.

However, we could not identify which factor(s) is/are responsible for HΔM and HΔM-SYK cells inhibition. Therefore, it remains to be clarified how basophils inhibit HΔM and HΔM-SYK cells at the molecular level.

(5) What are the critical MEIS1-target genes responsible for immune evasion?
We sought to identify the MEIS1-target genes responsible for the process of immune evasion achieved by H9M1 cells.
We performed microarray analysis comparing the RNA content of H9M1 cells with HΔM-SYK cells using in vitro cultured cells and in vivo inoculated for 1 week.
However, we could not identify genes specifically expressed by H9M1 cells involved in immune suppression. Therefore, how does MEIS1 protects H9M1 cells from basophils inhibition is still elusive.

Overall, this project demonstrated a basophil / T cells collaborative immune axis that detects and eliminates MEIS1-deprived leukemic cells. MEIS1 overexpression protects leukemic cells from this immune attack presumably by inducing immunosuppression of basophils through MEIS1 target genes.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関