Cancer stem cells (CSCs)/cancer-initiating cells (CICs) were first isolated in leukemia as leukemia stem cells [4,5]. In subsequent works, CSCs/CICs were successfully isolated from several solid malignancies. Colorectal CSCs/CICs were isolated using cell surface markers including CD133 by the ALDEFLUOR assay and as side population (SP) cells [6-9]. Colorectal CSCs/CICs exhibited greater tumor-initiating ability than that of the non-CSC/CIC population. Furthermore, colorectal CSCs/CICs showed resistance to chemotherapeutic reagents as was found for CSCs/CICs derived from other types of malignancies.

Feasibility of Colorectal Cancer Stem Cell Targeting Immunotherapy

Since colorectal CSCs/CICs are resistant to standard cancer therapies, they should be eradicated to cure the disease. Thus, CSC/CIC research has been focused on how to treat colorectal CSCs/CICs. Cancer immunotherapy might be a good candidate. The immune system is consists of antigen-specific acquired immunity and antigen-non-specific innate immunity. Several effectors have been shown to successfully recognize treatment-resistant colorectal CSCs/CICs, including cytotoxic T lymphocytes (CTLs) for acquired cellular immunity, antibodies for acquired humoral immunity, natural killer (NK) cells and γδT cells for innate immunity [10].

Cytotoxic T lymphocytes (CTLs)

CTLs are effector cells that are related to cellular immunity. CTLs recognize antigenic peptides presented by major histocompatibility antigen (MHC) class I molecule. Unlike NK cells and γδT cells, CTLs recognize target cells in an antigen-specific manner. Therefore, it is essential to express both MHC class I and antigens to be recognized by CTLs. In our previous study, CSCs/CICs derived as SP cells were shown to express MHC class I molecule at a level similar to that in non-CSCs/CICs isolated as main population cells [9]. Furthermore, colorectal CSCs/CICs express a TAA, CEP55. We compared the susceptibility to CEP55-specific CTLs of CSCs/CICs and that of non-CSCs/CICs and found that both colorectal CSCs/CICs and non-CSCs/CICs can be recognized by CEP55-specific CTLs at similar level [9]. Therefore, treatment resistant colorectal CSCs/CICs can be recognized by CTLs. In the following works, we found that colorectal CSCs/CICs express several TAAs including DNAJB8 and cancer/testis antigens [11,12]. These antigens are candidates for colorectal CSC/CIC-targeting immunotherapy.

Natural killer (NK) cells

NK cells are effector cells of innate immunity and they recognize target cells by ligands of natural cytotoxicity receptors and are not specific for antigens. A recent study has revealed that colorectal CSCs/
CICs isolated as sphere-forming cells express high levels of ligands of natural cytotoxicity receptors and that NK cells efficiently recognize colorectal CSCs/CICs [13].

γδT cells

γδT cells are T cell subpopulation that have distinct T cell receptors (TCR) consisting of a γ-chain and δ-chain, different from αβT cells, which have TCR composed of an α-chain and β-chain. The proportion of γδT cells in total T cells in epithelial lymphocytes is only 3% to 5% but, interestingly, γδT cells account for about 50% of cells in intestinal mucosa. Todaro et al. reported that zoledronate-activated Vγ9Vδ2 T cells efficiently recognize colorectal CSCs/CICs [14]. CTLs, NK cells and γδT cells kill target cells by secretion of cytotoxic granules that include perforin and granzymes. Therefore, these findings indicate that treatment-resistant colorectal CSCs/CICs are sensitive to apoptotic cell death induced by perforin and granzymes.

Antibodies

Antibodies are effector molecules of the humoral immune system, and several oncoprotein-specific monoclonal antibodies have been approved and are in clinical use for malignant diseases. Colorectal CSC/CIC-specific cell surface proteins can be targets of antibodies. Dallas et al. described that chemotherapy resistant colorectal CSCs/CICs induced by 5-fluorouracil (5FU) and oxaliplatin express a high level of insulin-like growth factor 1 receptor (IGF1R). Treatment with anti-IGF1R monoclonal antibody was shown to suppress the growth of colorectal CSCs/CICs both in vitro and in vivo [15]. A similar approach using anti-DLL4 monoclonal antibody has been reported [16]. Therefore, colorectal CSC/CIC-specific monoclonal antibodies can be candidates for colorectal CSC/CIC-targeting immunotherapy.

Perspectives of Colorectal Cancer Stem Cell-Targeting Immunotherapy

As described above, CTLs, NK cells, γδT cells and antibodies efficiently recognize and target colorectal CSCs/CICs. NK cells and γδT cells are non-specific effectors, and these effectors should recognize both colorectal CSCs/CICs and non-CSCs/CICs. Therefore, NK cells and γδT cells might be good candidates to treat evident tumor, since a tumor is composed of a large proportion of non-CSCs/CICs and a small proportion of CSCs/CICs. CTLs and antibodies are antigen-specific effectors, and they are candidate for specific eradication of CSCs/CICs. After induction of CTLs in vivo, some antigen-specific CD8+ T cells remain for a long time as memory T cells, and CTLs might thus be a good candidate for long-lasting immunization such as immunization for prevention of relapse. Antibodies are not affected by the immune status of the patient, and antibodies might thus be candidates for treatment of immune-suppressed patients.

Conclusion

Colorectal CSCs/CICs are resistant to standard cancer therapies, whereas they can be targeted by immunological effectors. Therefore, cancer immunotherapy combined with other standard cancer therapies is an attractive approach. Several cancer immunotherapies have already been approved and several clinical trials are ongoing. Therefore, it is expected that colorectal CSC/CIC-targeting immunotherapy and a protocol of combined standard cancer therapy and colorectal CSC/CIC-targeting immunotherapy will be established in the near future.

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