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ABSTRACT

Human body are losing several billions of cells every day. When cells are dying in vivo, the corpse of dead cell are not remained uncleared. Dead cells are efficiently recognized and cleared by multiple types of neighboring phagocytes. In the initial researches studying cell death, molecular mechanisms regulating cell death are more focused and the end corpse are simply considered as cellular debris. However, it has been revealed that various biological influences after cell death are important for the followed immune regulations. Healthy clearance of normal dead cells is silently carried out with immune tolerance. The exogenous or mutated antigens of malignant or infected cells can initiate the adaptive immunity inducing immunogenicity by adjuvant signals. Several pathogens and cancer cells have strategies to limit the adjuvant signals and escape immune surveillance. In this review, we present the overview of mechanisms of dead cell clearance and its immune regulations.

INTRODUCTION

An adult human body consists of 60 trillion cells conducting its roles in each tissues for living. The maintenance of body health needs the continuous changes of aged or damaged cells with newly generated cells. Aged cells after fulfillment, damaged cells, infected cells or malignant cells undergo the programmed cell death. The clearance of useless or damaged cells via programmed cell death is thought to be indispensable for maintaining homeostasis in living organisms (1). The proper clearance of apoptotic cell by phagocytosis is important for embryonic development, organ generation, tissue repairing, and appropriate immune response. Moreover, impaired clearance of dead cell has been considered as consequence or cause of the many diseases (2-4). Accordingly, the research interest of dead cell clearance is growing to understand its modulating networks, mechanisms and disease relevance.

In fact, approximately 150 billion cells (out of the 60 trillion cells in the human body) are dying every day (1). Interestingly, even though a number of cells are dead, experimental detection of dead cells is rare in histological studies of normal human tissues (5). And even in tissues (such as intestine, thymus, bone marrow, and lung) where turnover rates of cells are rapid, unremoved dead cells are hardly detectable. These observations suggest that homeostatic clearance of dead cells is operated at great degree of promptness and efficiency (4, 6, 7). Dying cells are recognized and internalized by professional phagocytes, such as macrophages and dendritic cells or neighboring non-professional phagocytes (8-10). Intraperitoneal injection of dexamethasone into mouse or exposure of ionizing radiation to whole body of mouse results in massive thymic apoptosis in thymus tissue. Within a few minutes after thymic cell death, the dead cells are swiftly phagocytosed by resident macrophages, and almost dead corpses are cleared in 24 hours (11, 12). And ischemia–reperfusion injury induces necrosis of renal epithelial cells in kidney tissues, but rapid scavenging of injured cells leads kidney regeneration and repair (13). Therefore, it is

considered that efficient and prompt phagocytosis of dead cells is the critical initial step for recovery of injured tissues. In the early studies, the phagocytic clearance of dead cells by phagocytes was thought to function simply in terms of dead cell debris scavenging. However, accumulated observations indicate that phagocytes after engulfing apoptotic cells can lead various biological consequences, depending on the engulfing situation, phagocytosed dead cell types, or phagocytic environment (14).

Here, we review the mechanism of apoptotic cell phagocytosis and biological response following dead cell clearance, such as immune tolerance, inflammation, tissue recovery, and homeostasis.

PHAGOCYTOSIS OF APOPTOTIC CELLS

Everyday several billion cells are dying in adult human body. Once cell death is occurred in a living organism, the dead cell corpses are immediately detected and cleared by phagocytes, such as macrophages and dendritic cells, rather than left in the body (15). It has been revealed that the mechanisms of dead cell clearance by phagocytes depend on the mode of cell death. But with regard to the mechanism of dead cell clearance following cell death, only the classification and analysis of apoptotic cases has been progressed. The molecular mechanisms by which phagocytes recognize and internalize apoptotic cells have been studied concentrically during last twenty years (16). In the previous studies, it has been revealed that phagocytes such as macrophages and dendritic cells recognize phosphatidylserines, which are externalized and positioned on the surface of dead cells (2). As phosphatidylserine functions as an indicating tag when apoptotic cells are subjected to be removed via phagocytosis by phagocytes, phosphatidylserines are initially called as "eat-me" signals (17). In normal and healthy cells, phosphatidylserines are unexposed on the cell surface because they are localized on the inner side of the surface membrane; however, when cells are dying,

phosphatidylserines are exposed to the surface of apoptotic cells. Annexin V is well known protein to bind phosphatidylserine in a specific manner. Therefore, Annexin V is usually used to identify the surface localization of phosphatidylserines in dying cells by flow cytometry analysis (18). Among several sequential phases of cell death event, apoptotic cells in early stage are found to be positively stained with Annexin V and negatively labeled with propidium iodide. As propidium iodide is used to detect DNA content by integrating into DNAs, early phased apoptotic cells shows the phosphatidylserine disclosure to apoptotic cell surface but not the increase of cell membrane permeability. So far the molecular mechanism exposing phosphatidylserine on the apoptotic cell surface had remained unclear. Several recent studies uncovered that flippases are involved in the asymmetrical distribution of phosphatidylserine in the surface membrane of healthy cell involves flippase. In a healthy cell, flippase enzymes function to have phosphatidylserine localize in the inner side of surface membrane via moving phosphatidylserine from the outer leaflet to the inner leaflet of the cellular plasma membrane. It has been reported that CDC50A and ATP11c play a role in the localization of phosphatidylserine in the cytoplasmic side of plasma membrane (19). It has been found that caspases cleave ATP11c upon cell death. This makes ATP11c inactive as an active flippase. In addition to the inhibition of flippase activation, it has also been found that when cells undergo programmed cell death, caspases also activate Xkr8 and Xkr8 plays a crucial role in effective transportation of phosphatidylserine from the inner leaflet of the membrane to the outer leaflet of the surface plasma membrane (20). It is now thought that the inactivation and activation of ATP11c and Xkr8 enzymes collaboratively trigger the exposure of phosphatidylserine on the outer leaflet of the surface plasma membrane. The exposed phosphatidylserine serves as a physical interacting recognition marker by macrophages. Phosphatidylserine was initially identified as the unique "eat-me" signals exposed on dying cells. Subsequently, a number of proteins have been found as phosphatidylserine-binding

receptors which is expressed in phagocytes including macrophages and dendritic cells. Several proteins of these, such as Milk fat globule-EGF factor 8 (MFG-E8), MerTK, and T-cell immunoglobulin mucin-3 (Tim-3) and T-cell immunoglobulin mucin-4 (Tim-4) are found to be critical for dead cell removal in vivo (21-23). Even though distinguished distinction in the roles of these proteins have not been still clarified in detail, it has been understood that distinctive proteins mediates the dead cell scavenging, depending on the types of phagocytes and organisms. Macrophages in the abdominal cavity phagocytoses dead cells mainly via Tim-4 and MerTK. Tim-4 induces dead cell phagocytosis via direct binding to the phosphatidylseine, an "eat-me" signal (24). MerTK mediates dead cell phagocytosis by binding to several mediating molecules such as growth arrest-specific gene 6 (Gas6) and protein S, both of which shows specific interacting activity with phosphatidylserine on the surface of dying cells. These two MerTK and Tim-4 proteins coordinately play essential roles in efficient phagocytosis of dying cells by macrophages resident in peritoneal region. On the contrary, inflammatory macrophages, which is generated by the intraperitoneal administration of thioglycollate, phagocytose dead cells in the dependent manner on MFG-E8 protein (21). In addition, splenic DCs express Tim-3 molecules and use Tim-3 as "eat-me" signal receptor to recognize phosphatidylserine-exposed apoptotic cells. The anti-Tim-3 antibody, which functionally blocks surface Tim-3 protein, blocks engulfment of dead cells by CD8^{+} DCs. The mitigated phagocytosis of dead cells subsequently results in reduced cross-presentation of dead cell-associated antigens (25). Depending on the different expressing pattern of these proteins on phagocytic cells, genetic mouse models targeting each molecule have shown distinct phenotype. MFG-E8 is mainly expressed in the germinal centers of the lymph nodes and spleen. Tangible body macrophages highly express MFG-E8 protein and phagocytose apoptotic lymphocytes. MFG-E8 knockout mice showed the inefficient phagocytosis of apoptotic cells by tangible body macrophages in vivo. On the

other hand, MerTK knockout mice was found to show the impaired clearance of thymic apoptotic cells (11).

On the other hand, it has been reported that proteins present on the surface of lk healthy living cells block the engulfment by phagocytes and render the living cells evade to be removed by phagocytosis. These are called as the "don't-eat-me" signals. CD47 protein is well-known "don't-eat-me" signal which has been reported to show phagocytosis-blocking activity (26). The anti-CD47 antibodies neutralizing surface CD47 has shown to trigger phagocytosis of even living cells in vitro and in vivo (27). Based on this phagocytosis-triggering activity of anti-CD47 antibodies, anti-CD47 antibodies was found to be effective to lead phagocytosis of cancer cells by macrophages (28). This anti-CD47 antibodies are under development as an immune modulatory anti-cancer drug.

For cells that have gone through apoptosis to be promptly phagocytosed by macrophages, it is indispensable that phagocytes migrate to dead cells. It has been found that dying cells secret a number of molecules such as chemo-attractants in order to recruit phagocytes. The releasing factors from apoptotic cells are referred to "find-me" signals. In several studies, it has been reported that ATP and lysophosphatidylcholine secreted from apoptotic cells play a critical role in recruiting phagocytes including macrophages (29). However, the molecular mechanisms of these "find-me" signal molecules recruiting phagocytes in vivo remains unclear.

DEAD CELL SCAVENGING IN VIVO

As dead cell clearance by macrophage occurs very rapidly and efficiently in the living organisms, dead cells are hardly observed left uncleared in the tissues. Although a significant number of apoptotic cells stained with TUNEL can be detected in thymus, apoptotic cells are mostly observed inside of resident macrophages (5). Based on this biological phenomenon,

this question has been asked; why dead cells must be removed immediately after cell death without remaining dead cell corpses? Using genetic mouse models deficient genes involved in the phagocytosis of dead cells by phagocytes, many studies have revealed the significance of apoptotic cell clearance in living organisms. In MFG-E8 and MerTK knockout mice, the in vivo phagocytosis of apoptotic cells shows impaired. Consequently, uncleared dead cell corpses induce the hallmark symptoms of autoimmune disease including significant elevations of serum anti-DNA antibody and anti-nuclear antibody titers (3). From these findings, dead cell clearance by phagocytes is thought to play a critical role in the maintenance of immune tolerance to self-antigen. This biological event has been understood as that phagocytes, by promptly clearing dying cells, prevent autoantigens from being released from dead cells and block the abnormal activation of autoimmune responses. On the other hand, several studies have been suggested another explanation that phagocytes might play an active role in inducing immune tolerance to self-antigen through the phagocytosis of apoptotic cells. The phagocytes that engulf apoptotic cells are thought to actively transduce negative or immune tolerating signals to self-reactive T cells by presenting engulfed autoantigens loaded on MHC. Multicellular organisms have dying cells everyday constantly for cellular turnover Apoptotic cells resident in specific tissues contain tissue-specific self-antigens. Therefore, these uncleared dead cell corpses could provide sources of self-antigens for antigen-presenting cells such as macrophages and dendritic cells. When dead cells in which apoptosis is induced in vitro are intravenously or intraperitoneally administered, T-cell responses to self-antigens generated from apoptotic cells are diminished (30). The splenic dendritic cells engulf administered apoptotic cells and self-antigen from apoptotic cells are presented to resident T cells for immune tolerance. As apoptotic cells itself could induce immune tolerance, although splenic dendritic cells are stimulated by activation signals such as costimulatory signals, the cross-presentation of self-antigen might result in removal of

self-antigen-specific T cells (31). Interestingly, under particular physiological conditions, the phagocytosis of apoptotic cells could lead the cross-presentation of self-antigens by phagocytes, resulting in the activation of T-cell responsive to the self-antigens. T cell activation against apoptotic cell-associated antigens has been broadly studied in the field of immuno-oncology. It has been reported that apoptotic cancer cells, either that apoptosis of tumor cells is induced in vivo or that apoptotic cancer cells are injected, could induce cancer cell antigen-specific T-cell activation (32). The efficiency of tumor vaccination using apoptotic cancer cells mostly depends on the type of cellular apoptosis in vaccinated cancer cells. It has been reported that disclosure of calreticulin protein on the apoptotic cancer cells efficiently leads anti-tumor immunity (33), suggesting that calreticulin may modulate phagocytosed tumor antigen-induced immune activation. In other studies, it has been observed that injection of stable cells artificially expressing CD1d, which can load α-galactosylceramide and tumor antigens prompt tumor immunity (34). In this studies, CD1d expressing cells were understood to undergo apoptosis in vivo and are phagocytosed by dendritic cells. The engulfed dendritic cells showed tumor antigens by cross-presentation in order to activate tumor antigen-specific cytotoxic T lymphocytes. This activation of cytotoxic T cells by tumor antigen-presenting dendritic cells was cooperatively achieved by activated natural killer T cells. The detailed molecular mechanisms about the immune response to apoptotic cell- or dead tumor cell- associated antigens need to be elucidated. The studies for mechanisms will provide new insight for controlling phagocytosed apoptotic cell-induced immune responses and cancer immune therapeutic strategies.

VARIOUS TYPES OF PHAGOCYTES FOR DEAD CELL ENGULFMENT

In living organisms, professional phagocytes such as dendritic cells and macrophages mainly play an engulfing role and control the following immune responses. In particular,

classification of types of phagocytes has being studied to uncover the actual player contributing to immune modulation. Each tissue has the tissue-specific phagocytes including macrophages and dendritic cells. These tissue-resident phagocytes internalize the apoptotic cells. Recent studies have found that the distinct functions for immune modulation of phagocytes depend on tissue specificity and different subpopulations of phagocytes even in same tissue. In tissues, phagocytes have been found as heterogeneous types showing functional diversity (35). In other words, particular subpopulation of phagocytes is mainly responsible for dead cell clearance in tissues. In fact, several populations reside in the lymph nodes and spleen have been found to play a critical role in the phagocytosis of dying cells, also in followed immune tolerating modulation. Like discussed before, the intravenously administrated apoptotic cells do not activate immune response to apoptotic cell-associated self-antigens and result in immune tolerance. For instance, particular subpopulation of macrophages and/or marginal metallophilic macrophages reside in marginal region of spleen. Since the marginal zone of the spleen is the site in which bloodstream passes, splenic marginal zone macrophages have been found to phagocytose apoptotic cells in the blood. The crucial role of marginal zone macrophages in the dead cell phagocytosis and the tolerance induction has been observed in the CD169-DTR mice. In CD169-DTR mice, marginal zone of macrophages was specifically deleted by DT injection (30). Dendritic cells expressing CD103and CD11care also resident in the splenic marginal zone. These dendritic cells contribute to cross-presentation of antigens originated from apoptotic cells to CD8-positive T cells. This suggests that cooperative immune modulation by dendritic cells and macrophages in the (36). Apoptotic cells induce immune tolerances during dying processes. The underlying molecular mechanisms of immune tolerance have been reported. When apoptotic cells were injected into mouse by intravenous injection, metallophilic macrophages in spleen were induced to express CCL22. CCL22 expression in splenic macrophages lead recruitment and

inducetion of FoxP3-positive regulatory T cells (Treg cells). In the case of cancers, On the other hand, a number of apoptotic cells in tumor tissue can result in immunological activation in response to tumor cell-related antigens and develops anti-cancer immunity targeting tumor cells. The subsets of dendritic cell and macrophage involved in anti-cancer immune activation have been characterized. When mice are subcutaneously injected with dead tumor cells, CD169-positive macrophages in the lymphatic cavity of lymph node phagocytose apoptotic cells or dead cell debris in the lymph flow. The dead-cell engulfed macrophages led immunological activation in response to neoantigens. CD169-positive macrophages consist of two subsets; CD11c-negative and CD11c-positive macrophages. CD11c-positive macrophages in the border region between nasal cavity and T cell zone present neoantigen originated from dead tumor cell to CD8 positive T cells by cross-presentation. Given that immunological activation against apoptotic cell-related antigens are important for the treatment of cancer and autoimmune diseases, the characterization of macrophage subpopulations involved in dead cell antigen-responsive macrophages are essential research subjects.

During last a few decades, cell death had been thought as the final event of a cellular life cycle, and dead cell corpses had been considered only as the debris which is simply to be removed by phagocytes, such as dendritic cells and macrophages. In many recent studies, it has been clarified that cell death process induces various subsequent biological events indicating that cell death serves as a starting point. And it has also been revealed that dying cells actively release physiological substances which activate several biological processes. Among the physiological responses triggered by cell dying, one of the well characterized events is inflammation. High mobility group box protein 1 (HMGB1) is a dead cell-releasing factor which stimulate immune response. HMGB1 protein was initially identified as a nuclear factor. However, nuclear HMGB1 is also released when cells are dying via non-apoptotic

death (37). Once HMGB1 is released, HMGB1 proteins results in inflammatory responses by targeting macrophages and dendritic cells (38). C-Type Lectin Domain Family 4 Member E (CLEC4E) Mincle, a C-type lectin, is expressed on the surface of macrophages. CLEC4E receptor recognizes soluble factor, SAP130 released from dying cells in order to lead inflammation (39). The inflammation-inducing factors which is originated from dying cells refer to damage-associated molecular patterns (DAMPs). Additionally, in many lines of studies, it has been uncovered that substances derived from dying cells contribute to tissue repair and organ regeneration. When liver cells get damages such as oxidative stress, dying hepatic cells have been known to release IL-11. IL-11 affects on neighboring normal cells and lead regeneration of liver tissue. Several studies have reported that semaphorin 3E is highly expressed in apoptotic hepatic cells and modulates liver fibrogenesis, repair and regeneration (40). Furthermore, other studies have demonstrated that chronic liver damage induces dead cell phagocytosis and upregulation of Wnt3a in macrophages, resulting in liver regeneration. Even though the significant association between cell death and regeneration of injured tissue has been found so far, the specific molecular mechanisms have not been elucidated. Therefore, the further progress of research finding the detailed mechanisms of dead cell-induced tissue regeneration is highly expected.

APOPTOTIC and NON-APOPTOTIC CELL DEATH

During last a few decades, cell death process has been intensively studied. The programmed cell death (called apoptosis) was considered to be the major type of cell death which is usually occurring in vivo. Apoptosis is companied with activation of caspase cascade signaling and the eventual degradation of intracellular substrates. Therefore, apoptosis is considered as an active type of cell death which is regulated by active molecules. Given that caspases are activated in apoptotic cells, detecting active caspases has been widely used as

the analysis of apoptotic cells is Additionally, terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay has also been well established for detecting apoptotic cells in vitro and in situ (41). By using these assay technique, programmed cell death could have been detected in multiple organs during embryonic development stage and normal tissue turnover. The high technique to detect apoptotic cells in vivo in the real time has been developed. Based on this assay technique, the dynamic turnover of apoptotic cells in vivo and its effect on the neighboring cells are intensively characterized especially occurring during embryogenesis (42). Non-apoptotic cell death (called necrosis) is a type of cell death, which is caused by other physical stimuli or heat. And pathological cell death caused by diseases is also considered as a passive mode of cell death. The necrotic cell death shows the particular morphological characteristics of dying cells. Given that the necrotic cell death is a passive type of cell death, it was thought that no particular molecular mechanisms exist in necrotic cell death. Recent studies have found that necrotic cell death has several subtypes, which are regulated by active molecules. Cell death controlled by receptor interacting serine/threonine kinase 1 (RIPK1) / receptor interacting serine/threonine kinase 3 (RIPK3)- and mixed lineage kinase domain-like (MLKL) has been identified. These subtypes of necrosis are referred to necroptosis (43). As another new type of necrotic cell death, caspase-1-mediated cell death is identified as pyrotosis (44). These novel subtypes of non-apoptotic cell deaths exhibit the typical necrotic morphological feature. However, necroptosis or pyroptosis was found to contribute to pathological feature of various diseases. More recently, additional type of cell death referred to ferroptosis, during which iron ions are required, has been identified (45). The molecular mechanisms of newly identified cell death have been studied in the cultured cell level in vitro based on the features of cell death phenotype and activated signaling molecules. Although the in vivo role of the associated molecules have also been studied using genetically modified mouse models, the physiological function of

new cell death events have gradually elucidated based on their significances. As there is no satisfied method to detect the new types of cell death so far, the in vivo study of new cell deaths is having obstacles. It is considered that the mechanisms of dead cell removal by phagocytes depend on what type of cell death is occurring. Moreover, dendritic cells and macrophages could decide the appropriate responses to dead cells, depending on the specific mode of cell death. In order to follow the physiological and pathological consequence of dying cell in vivo, it is necessary to investigate how dendritic cells and macrophages response to dead cells according to the different modes of cell death.

INNATE IMMUNE CHECKPOINT FOR CANCER THERAPY

Recently, T cell immune checkpoint inhibitors for cancer therapy has achieved great outcomes for successful cancer treatment. However, the effect of T cell immune checkpoint inhibitors is very limited in only a population of patients. Patients not responding to these drugs show non-T cell-inflamed tumors. And the removal of cancer cells by T cells is significantly dependent on activation of innate immunity. Therefore, additional targeting innate immunity may be a strategy for developing improved therapies against cancer. The TAM receptor tyrosine kinases including TYRO3 protein tyrosine kinase (TYRO3), AXL receptor tyrosine kinase (AXL), and MER receptor tyrosine kinase (MERTK) have been importantly studied as innate immune checkpoint proteins involved in critical steps of anti-tumor immunity (46). Efferocytosis refers to the process by which dead cells are cleared by phagocytes. TAM receptor tyrosine kinases have been known to inhibit the activity of dendritic cells which is one major professional phagocytes and dysregulate the generation of chemokines, eventually resulting in the immune evasion of tumor cells. Therefore, TAM signaling blockade strategy for anti-cancer immune therapy is being highlighted to enhance the cooperative effect with anti-tumor adaptive immunity and immune checkpoint blockade

targeting T cells.

Current immune checkpoint inhibitors such as anti-PD-1, anti-CTLA-4, or anti-PD-L1 antibodies show excellent promising outcomes in anti-tumor effect in melanoma and lung cancer patients (47). Moreover, modulating strategy of immune cells has led the long-lasting anti-tumor immunity. However, the limited population of patient is showing responsive to T cell immune checkpoint therapy. The studies understanding the distinct mechanisms between responsive and unresponsive patients are intensively continuing. In order to overcome the narrow response to T cell immune checkpoint therapy, co-treatments with multiple reagents have been applied. For example, combinational therapy with rituximab and anti-PD1 antibody for follicular lymphoma showed the 66 % response rate. Although this therapeutic outcome is a remarkable for some patients, still a large portion of patients have restriction to use immunotherapies. Unresponse to T cell immune checkpoint inhibitor is mostly caused by a loss of T cell response in tumor tissue. Therefore, it is important to target other novel immune inhibitory pathways that can circumvent the failure to T cell immune checkpoint therapy. Several pathways have been considered as targeting candidate. The alternative therapy targeting new candidates should not have the collateral damage to the patient. The blockade of receptor tyrosine kinases (RTKs) can be achieved by small molecule inhibitors, ligand blockers, and other inhibitory strategies. Recently, new cancer therapeutic strategy targeting the TAM tyrosine kinase receptors has attention and a number of small molecule inhibiting TAM receptors are in development (46). A small molecule inhibitor for AXL, BGB324 showed significant regression of the growth of multiple cancer cell lines. BGB324 also inhibited migration, metastasis, and angiogenesis in breast cancer xenograft mouse model. Based on the preclinical effects, the following Phase 1trial was finished. In mice tumor model system, it has been found that activated AXL tyrosine kinase leads resistance to erlotinib, a tyrosine kinase inhibitor (TKI) targeting epidermal growth factor receptor (EGFR).

Drug sensitivity to erlotinib TKI was increased upon genetic deficiency or small molecule inhibition of AXL. EGFR-mutant lung cancer samples obtained from patients with acquired resistance to TKIs showed increased expression of AXL protein level was found to be increased in mutant EGFR lung cancer tissues isolated from patients resistant to TKI cancer therapy. Several MerTK-specific small molecule inhibitors are under development. In mouse models for non-small-cell lung cancer (NSCLC), UNC2025, the MerTK small molecule inhibitor was showed significant suppression of tumor growth. UNC2025 sufficiently induced death of NSCLC tumor cells carrying oncogenic mutations such as k-Ras and EGFR mutations (48). Another MerTK inhibiting small molecule UNC1666 is also in development. Even though MerTK inhibitors targets other proteins such as fms related tyrosine kinase 3 (FLT3), this targeting fortunately led the supportive anti-tumor effect. Ligand trapping and monoclonal antibodies are considered as an alternative method. A GAS6 high affinity engineered extracellular domain of the AXL tyrosine kinase receptor could capture the ligand not to function through the AXL-mediated sequestering of GAS6. In the similar manner, the soluble ligand-binding protein fragment of MerTK functioned as a destrupting molecule for GAS6, leading the impaired engulfment of apoptotic cells by macrophages. Monoclonal antibodies targeting TAM receptors have been reported. TAM receptor tyrosine kinase on cancer cells can induce survival and transformation. And their activities of MerTK affecting on innate immune response can help immunological activation. Therefore, TAM receptor tyrosine kinases are good targets for pharmacologic. An anti-tumorigenic function of TAM signaling pathway has been found in colon carcinoma model induced by inflammation. Several studies have observed that the outcome of TAM inhibition can be different depending on the severity of inflammation, the amount of TAM RTK-expressing cells, and the phase of tumor progression. Studies using TAM tyrosine kinases inhibitors will reveal the detailed mechanisms that TAM signaling contributes to tumor killing. In order to evaluate TAM

inhibitors as an anti-tumor therapeutic drug, all the important factors resulted by systemic blocking of TAM inhibitors should be considered; the long-period effects, the degenerative degree of photoreceptor and vision, extratumoral homeostasis. New cancer immunotherapeutic strategy targeting innate immune checkpoints might trigger recruitment and activation of immune cells in patients.

IMMUNOGENEIC CELL DEATH

The concept of immunogenic cell death has been generated in tumor biology. Adjuvanticity of immunogenic dead cells is detected by the unique damage-associated molecular patterns. This simplified characterization of molecular phenomenon is based on immune surveillance against primordial system. Therefore, the concept of immunogenic cell death could not be applied to anticancer immune surveillance. In the context of microorganism infection, it has been found that viruses and bacteria develop various strategies to evade immune surveillance by shading exposes or recognition of damage-associated molecular patterns. The molecules which multiple microorganisms are using for immune escapes include orthologues of anti-apoptotic proteins such as BCL□2 family, negative regulating proteins of autophagy, dephosphorylating proteins of eIF2A, inflammasome-inhibiting proteins, and/or blocking factors of type I IFN signaling, with the aim of overting microorganisms pathogenicity. Once host is infected by pathogens, host determines the adjuvanticity of infected cell death. Adjuvanticity rather than antigenicity controls immunogenicity. Malignant transformation of cells also leads selective adjuvaticity in patient and high adjuvaticity help successful cancer immunosurveillance. Once neoplastic cells escape immune detection and removal, malignant cells develop to tumor. This might be caused by the inhibition of damage-associated molecular patterns exposure and recognition, the subsequent immunological responses. Even though advanced cancers show a high antigenicity, the malignant tumor cells exhibit various

mechanisms to make immunogenicity lower. Based on their escaping mechanisms, cancer cells impair the efficiency of multiple anti-cancer therapy including chemotherapy, radiotherapy and immunotherapy. Therefore, restoration of the immunogenicity of cancer cells might reconstitute the adjuvanticity, eventually achieving therapeutic success (49).

CONCLUSIONS

Here, we overviewed the molecular mechanisms of apoptotic cell phagocytosis and its pathophysiological significance. So far, the mechanisms of cell death, classification of subtype of cell death, disease relevance of cell death are intensively studied. Based on the recent accumulated observation indicating the significance of 'post-apoptotic biological events', the interest of studying dead cell clearance and the subsequent biological consequences is growing. To date, to understand physiological role of dead cell clearance, we need to overcome a number of challenges, such as the identification of various cell death types and the development of analytical methods for determining their biological significance and the modulating mechanisms of physiological response after cell death and dead cell clearance. As the subsequent biological events after dead cell clearance depends on the cell death modes, types of engulfing phagocytes, tissue specificity, and phagocytic microenvironment, the precise understanding about dead cell clearance will be important. The detailed verification about the biological role of dead cell clearance will provide deep knowledge to understand the causes and consequences of impairment of dead cell clearance in multiple human diseases.

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