

How Phosphatidylserine Is Exposed on Tumor Cells Determines The Tumor's Microenvironment and the Success or Failure of Checkpoint Therapy

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Research Article

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Abstract

One constant in all malignant somatic tumors is their continuous growth and it is hypothesized that there are only two types of cell mutations that can cause this and that their microenvironments are determined by how those mutated cells eventually die and by how phosphatidylserine (PS) is exposed on their surface. When a mutation in a cell causes its rate of mitotic cell division to *continuously* exceed the rate necessary for its replacement after its programmed cell death (PCD) a continuously growing tumor will form and all of the tumor cells will expose PS by the Xkr8 transmembrane scramblase molecule when they die causing an inflammatory and immune suppressive microenvironment. When a mutation in a cell eliminates PCD a *continuous* tumor growth begins because all those cells will continue dividing until they die a senescent death where PS is exposed by the TMEM16F scramblase molecule and causes an inflammatory microenvironment. Inflammation stresses somatic cells to expose checkpoint molecules (CPMs) on them and on immune cells that could potentially eliminate them. Only in tumors where PCD has been eliminated will CPM be exposed and only in them will immune checkpoint inhibitors (ICIs) be effective.

Introduction

One common factor in all malignant tumors is their continuous growth and there appears to be only two kinds of mutations that can produce this. In one the mutation continuously increases the rate of mitotic cell division of a cell above the rate required for its replacement after its programmed cell death (PCD). In the other the mutation eliminates PCD and the mutated cell and its progeny continue dividing until their senescent deaths. The microenvironments of these two tumors will be different because of the way PS moves to their surface. PS is present in cell membranes where it is kept on their inner leaflets in an energy dependent manner but it moves to their surface when cells die by PCD and when they are damaged or stressed [1-3]. Cancer cells that die by PCD will expose PS by the Xkr8 scramblase molecule [4] and the cells in tumors where PCD has been deleted will die a senescent death where PS is exposed by TMEM16F [5]. PS exposed by Xkr8 on cells dying by PCD will suppress inflammation and immune cell activation and the cells will be rapidly removed by phagocytes that recognize PS [4]. Billions of cells die by PCD each day and phagocytes remove them so efficiently that PS can't normally be detected [6]. The microenvironments of tumors whose cells die by PCD where PS is exposed on Xkr8 will be inflammation and immune suppressive [4]. In cancers where PCD has been deleted all the cancer cells will die a senescent death and PS will be exposed by TMEM16F where inflammation will be generated, immune cells will be activated and blood coagulation will be present [5]. Though immune cells are activated in tumors where PS is exposed by TMEM16F, the inflammation that the PS generates will activate CPMs on the tumor cells and on activated immune cells that can phagocytize or kill them [7,8]. In the following we will, in a necessarily very brief and cursory manner, examine PS's involvement in inflammation, immune cell activation, blood coagulation and CPM exposure.

Inflammation

Inflammation physiologically stresses cells to expose PS in order to amplify and accelerate immune responses and blood coagulation. Anytime cells are damaged or stressed calcium enters them, PS is exposed on TMEM16F, inflammation begins and is then accelerated when the PS binds to the TIM-1 receptor on Th1 immune cells and activates its feedback generation of inflammatory cytokines that stress somatic cells to cause the exposure of more PS [9]. PS exposed on damaged and inflammation stressed somatic cells will signal for their removal but in order to allow those cells not lethally damaged to recover when the damage has been controlled the inflammation also exposes CPMs on the somatic cells to protect them and on activated immune cells that could eliminate them [7,8]. The Th1 cell's ability to initiate and amplify inflammation was discovered in 2017 when it was found that a lethal inflammatory storm was produced in Ebola infected mice when PS on the Ebola virus would bind to the TIM-1 receptor on Th1 immune cells [9]. The Ebola infection was repeated with TIM-1 knockout mice and the mice survived and the inflammatory storm was prevented even though the viral load was only minimally reduced [9]. The inflammatory storm produced by PS in an Ebola infection is a pathologic manifestation of a physiologic response that becomes pathologic in Ebola because it is a long linear enveloped virus with PS exposed all over its surface. The physiologic amplification of PS exposure by Th1 cells becomes pathologic in conditions like severe septicemic infections and massive trauma when huge numbers of PS molecules are exposed.

Immune response

Immune cells receive a wakeup call anytime cells are damaged and PS is exposed by TMEM16F. The TMEM16F exposed PS binds to the tyrosine kinase TIM and TAM receptors on immune cells and activates their secretion of cytokines in the innate immune response. These cytokines direct the activation or suppression, proliferation, differentiation, orientation and mobility of immune cells in both the innate and the adaptive immune response but the adaptive response only takes place when pathogen associated molecular patterns (PAMPs) are recognized by toll like receptors (TLRs) on macrophages and dendritic cells and they begin secreting inflammatory cytokines. The dendritic cells phagocytize pathogens and MHC present their peptides to complementary MHC peptides on T cells and they MHC presents those peptides to a MHC complementary peptide on B cells "that looks like the pathogen's peptides" and those B cells secrete antibodies with Fab peptides complementary to pathogen peptides. The innate immune response is essentially the executive branch of an immune response that directs the work force dendritic, T and B cells to produce an antibody product. There are a small number of immune cells that must produce a very large number of cytokines and this is made possible, in part at least, by three kinds of TIM and of TAM receptors. This makes it possible for an individual immune cell to produce at least six different kinds of cytokines and when more than one receptor is on an immune cell their numbers may increase exponentially. Immune cells with TIM receptors primarily activate the cytokine secretion of cells as is demonstrated by the TIM-1 receptor on Th1 immune cells [9] while TAM receptors primarily suppress cytokine secretion as is demonstrated by the TAM receptor on Th2 immune cells [8]. The immune cell's interactions have been developed over time by evolution with the elimination of mutated immune cells whose cytokines decrease survival while retaining those that increase it.

Blood coagulation

When cells are damaged tissue factor (TF) is exposed along with PS ensuring that blood coagulation and immune activation always coexist. Prothrombin must be changed to thrombin in order for blood coagulation to take place and TF initiates thrombin generation [10] and its generation is amplified by the coagulation cascade where PS functions as the cascade's platform [11,12]. TF exposed by cell damage binds to activated factor VIIa and the TF/VIIa complex activates factors IX and X. Activated factor Xa changes prothrombin to thrombin and it directly or indirectly activates all coagulation components including those necessary for the coagulation cascade. Activated factor IXa is a rate limiting component of the cascade that must be present for its thrombin generation. TF can't activate enough of factor IXa for the cascade's rapid thrombin generation so cascade generated thrombin activates factor XI and it activates factor IX. Actually this mostly maintains cascade thrombin generation because cascade thrombin generation and factor XI activation are codependent. The cascade can only generate enough thrombin to produce hemostasis when factor XII can be activated and this can't happen until a vascular wall is breached and collagen is exposed. Factor XII is activated when it binds to sulfatide on activated platelets [13] where it binds to factor XI so that it can activate factor IX. Fortunately for us factor XII activation can't happen intravascularly because activated platelets secrete a factor XII activation inhibitor [14]. When a vascular wall is breached collagen is exposed and activated platelets with sulfatide and PS exposed on their surface bind to the collagen and blood flow washes the inhibitor away so that factor XII can bind to the sulfatide enabling the cascade's exponential thrombin generation specifically where it's needed [14].

Checkpoint molecules

When cells are damaged or stressed calcium enters them and PS is exposed on TMEM16F causing inflammation to begin and it physiologically induces CPMs on those cells and on activated immune cells that could remove them [7,8,15,16]. When the damage is controlled calcium leaves the cells, the inflammation stops and CPMs will disappear and the cells not lethally damaged and the inflammation stressed PS exposure is no longer needed, those cells can potentially recover. Those physiologic CPMs produce pathologic consequences in somatic tumors with inflammatory microenvironments where they protect tumor cells. The administration of immune checkpoint inhibitor (ICI) monoclonal antibodies removes the tumor cell's protection. Unfortunately those ICIs also remove the protection of cells damaged by the reactive oxygen species (ROS) produced by mitochondrial energy generation [17,18]. The continuous presence of ICIs and the continuing ROS generation can eventually produce pathologic consequences. Somatic tumors where the tumors die by PCD and PS is exposed on Xkr8 are naturally immune and inflammation suppressive and the administration of ICIs to them will only produce pathologic side effects.

In Conclusion

If it's true that how PS is exposed on tumor cells produces two basic tumor types, those with and those without inflammatory microenvironments, then the administration of ICIs will only be effective in those with inflammation. This hypothesis can be tested in animals with tumors by determining their tumor's inflammatory status and then administering ICIs to both to determine their responses.

Abbreviations

Phosphatidylserine (PS), Tissue factor (TF), toll like receptor (TLR), programmed cell death (PCD), checkpoint molecules (CPMs), immune checkpoint inhibitors (ICIs), pathogen associated molecular patterns (PAMP)

Declarations

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