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The role of eicosanoids in cancer and inflammation

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THE ROLE OF EICOSANOIDS IN CANCER AND INFLAMMATION

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ABSTRACT

Inflammation is the body's natural response to injury or infection by pathogens [73]. Chronic inflammation is a hallmark of cancer and may act to exacerbate tumor growth and metastasis[116]. The resolution of inflammation is now known to be an active process mediated by small lipid autacoid molecules termed specialized pro-resolving lipid mediators (SPMs)[73,90]. Chronic inflammation can result in a vicious cycle of tissue injury, inflammation, and further tissue injury. We evaluated the SPM, resolvin E1 (RvE1), to stimulate the resolution of inflammation in multiple murine models of pancreatic cancer and metastasis. RvE1 mediated macrophage class switching in the tumor microenvironment, increased immune cell infiltration, and improved immune resistance[116]. On the macroscopic scale, RvE1 treatment inhibited tumor growth and number of metastases. Notably, multiple studies showed that RvE1 improved anti-tumor activity of current frontline cancer treatments such as chemotherapy (e.g. cisplatin and gemcitabine) and immunotherapy (e.g. anti-PD1). There was no observed toxicity associated with RvE1 as both a monotherapy and in combination with other treatments. These results show the efficacy of RvE1 in

enhancing the resolution of inflammation within the pancreatic cancer microenvironment and suppressing tumor growth. The current study provides a robust platform for conducting further pre-clinical investigations for SPMs in the treatment of different cancer types.

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
APC	Antigen Presenting Cell
BU	Boston University
COX	Cyclooxygenase
DAMPs	Damage associated molecular patterns
DC	Dendritic Cell
IFN	Interferons
IL	Interleukin
LPS	Lipopolysaccharide
LX	Lipoxins
Mar	Maresins
NETs	Neutrophil Extracellular Traps
NK	Natural Killer Cell
NLR	Neutrophil to Leukocyte Ratio
NSAID	Non-steroidal Anti-inflammatory Drug
PAMPs	Pathogen associated molecular patterns
PGE	Prostaglandin
PRR	Pattern recognition receptors

PUFA	Polyunsaturated Fatty Acid
Rv	Resolvin
SPMs	Specialized Pro-Resolving Mediators
Th	T Helper
TLR	Toll-like Receptor
TME	Tumor Microenvironment
TNF	Tumor necrosis factor
VCAM	Vascular Cell Adhesion Molecule

INTRODUCTION

A common phrase in the field of the hallmarks of cancer physiology is "if genetic damage is the match that lights the flame of cancer, then inflammation is the fuel that feeds the flames".[1] The current view of inflammation involves complex changes in the host microenvironment that include but are not limited to influx of chemokines and cytokines, activation of inflammatory cells (e.g. macrophages, dendritic cells, and T lymphocytes), and restructuring of the vasculature near the site of injury. However, inflammation has long been understood as an indicator of pathogenesis and disease since before the beginning of the Common Era when the Roman writer Aulus Celsus noted what would become the cardinal signs of inflammation; calor (heat), rubor (redness), tumor (swelling), and dolor (pain). Another Roman researcher, Galen, later described a fifth sign of inflammation, loss of tissue function [2,3]. Taken together these findings built the foundation for later works by influential inflammation researchers such as Gaubius, Metchnikoff, and Wagner.[2,4,5]

Research into inflammation exploded with the invention of the compound microscope by Zacharias Janssen in 1590 and later improved by Leeuwenhoek in 1790[4,5]. Microscopes provided researchers with a tool to visualize the activity of inflamed tissue and propose new mechanisms for the activation and effects of

inflammation on the host tissue and microenvironment. Thanks to the compound microscope the mechanisms behind the five cardinal signs of inflammation were elucidated. Beginning with Herman Boerhaave in the early 18th century scientists began viewing the signs of inflammation, such as the size of blood vessels not being big enough to carry enough blood to inflamed tissue causing the extra blood to generate friction and cause calor (heat) [3,4,6].

A diverse knowledge of all involved cell types must first be established to understand inflammation. When researchers first observed blood under a microscope, they only noted the red blood cells, and so were focused on them. It wasn't until William Addison and Gabrielle Andral in the late 19th century identified white blood cells and Paul Ehrlich classified their morphogenesis that the steps towards immunity research could be taken. Together with Ehrlich, Ilya Metchnikoff described white blood cells and their role in phagocytosis through Metchnikoff's insect embryology studies. They would go on to share the 1908 Nobel prize in medicine and physiology for their discoveries on immunity and phagocytosis.[5-8]

Inflammatory Activation and Mechanism

The identification of white blood cells and the mechanism of phagocytosis opened the doors for more exciting research into host immunity and its response to injury, disease, and inflammation. While inflammation is established as a host defense mechanism, the manner in which it is activated and the constituent cell types and pathways that act on the host tissue differ between inflammatory stimuli. Two important pro-inflammatory signaling mechanisms are mediated by damage associated molecular patterns (DAMPs) or pathogen associated molecular patterns (PAMPs).[9,10,16,17] PAMPs are structures of microbes that are recognized by immune and non-immune cells causing the activation of pattern recognition receptors (PRR).[9,16] Examples of PRRs include Toll-like receptors (TLRs), RIG-1, and NOD-LRR.[17] These receptors then activate the full inflammatory cascade with production of chemokines and cytokines as a chemotactic gradient for recruitment of immune cells. DAMPs are endogenously produced warning signals of cell stress or injury. They are released in the intra- and extracellular space for the purpose of inducing non-infectious inflammation for the amelioration or destruction of harmful stimuli.[9,12-14]

The pattern recognition system is a part of the innate immune system. Once PAMPs or DAMPs are identified by TLRs or other recognition receptors, the activated TLRs then induce intracellular signaling cascades.[11,12,16]

Downstream of TLRs, transcription factors enter the nucleus to change expression levels of pro-inflammatory cytokines. Nf-κB is one of the most well studied transcription factors that plays a key role in inflammation and cancer. This pathway is activated in macrophages after PAMP signaling and induces increased expression of cytokines through direct modification of DNA translation. [18,19] Release of cytokines such as Interleukin (IL)-1β, Interferons (IFN), and others act as signals for immune cell migration, differentiation, and proliferation.[20-22] These cytokines are responsible for the protective functions of inflammation and yet are also the cause of much dysfunction in the body when dysregulated. Dysregulation of inflammatory pathways can lead to chronic inflammation and further tissue damage.[73]

The harmful dysregulation of cytokines represents a shift away from homeostasis. A host of factors can lead to dysregulation of cytokines including overproduction of pro-inflammatory cytokines and diminished pro-resolution and anti-inflammatory pathways.[24] It has been shown in recent studies that during cases of chronic inflammation, such as obesity, certain cytokines such as IL-6, TNF-α, IL-10, and IFN-γ were all found to be upregulated.[21,24] It has also been noted that, because there is redundancy in cytokine pathways, irregular levels of inflammatory cytokines can perpetuate the chronic inflammation due to

crosstalk between the cytokines and influence the cellular environment to maintain a highly inflammatory setting.[25] Cyclooxygenase-2 (COX-2), an enzyme that mediates chronic inflammation, is activated in response to inflammation and the production of pro-inflammatory cytokines.[26,27,30] If cytokine levels stay above the homeostatic level, COX-2 can cause an overproduction of lipid mediators termed prostaglandins (PGE) that potentiate the inflammatory response to a chronic state. These mediators act on inflammatory cells to increase expression of inflammatory cytokine receptors thereby inducing differentiation of T helper (Th) cells.[28,29] When improperly regulated, these T helper cells; specifically Th1, Th2, and Th17, can result in chronic inflammatory diseases.[31]

Chronic Inflammation

Chronic inflammation is a hallmark of many common diseases that men, women, and children alike can suffer from. Chronic inflammation occurs either when the harmful inflammatory stimulus is present for extended periods of time or there is a disruption of the resolution of acute inflammation.[50] Asthma, rheumatoid arthritis, psoriasis, diabetes, cancer, and other diseases involve chronic inflammation as the primary pathology or an aggravation of previous

symptoms.[31,32,47] In addition to Th cells, macrophages play a critical role in the inflammatory process and consequently in the development of chronic inflammation. Despite their beneficial effects in most cases, these immune cells can become pathogenic when not regulated correctly by the available cytokines. In this way, inflammatory cells and inflammation in general can be equated to an open flame under a skillet. Suppression of cytokines or a lack of immune cell activation would do little to protect the host when an inflammatory response is necessary similar to how low heat from a burner would not cook the desired item fully through or would take a significantly longer time to achieve the same effect as proper heating. On the other hand, having the heat too high would result in a charred, damaged piece of food that would likely not taste the same as when cooked correctly, just as the inflammatory response can result in tissue damage and fibrosis in states of chronic inflammation[128].

The mechanisms of chronic inflammation are still under investigation and there are a plethora of studies on the matter. However, the effect of dysregulated cytokines on both innate and adaptive immune cells has been intensely studied. Class switching in macrophages, decreased efferocytosis, and increased apoptosis are factors that can initiate chronic inflammation.[47,48] Tissue damage indicative of chronic inflammation is mediated by increased granulocyte

penetrance and neutrophil extracellular traps (NETs).[49] While necessary in both chronic and acute inflammation, neutrophils and macrophages both play important roles in the pathogenesis of chronic inflammation. Their ability to phagocytose ("pro-resolution") harmful debris and produce large amounts of cytokines makes them necessary but also key points of dysfunction when inflammation refuses to subside.

Neutrophils are the first preventative mechanism to act directly at sites of inflammation following chemotactic gradients.[50] However, increased expression of certain factors such as adhesion molecules, e.g. vascular cell adhesion molecules (VCAM) that neutrophils and other immune cells use to migrate to the site of injury results in an endless cycle of inflammation. Neutrophils act to clear harmful debris from the inflammatory site and at the same time generate cytokines that can be both beneficial and detrimental, for instance, by recruiting additional VCAMs. Additionally, neutrophils can initiate apoptosis in damaged cells. [49,52,53,58] Apoptosis then causes the release of additional pro-inflammatory cytokines that recruit inflammatory leukocytes.[50,] Interestingly it has been discovered that there is a strong interplay between macrophages and neutrophils during bouts of chronic inflammation. Tissue resident macrophages normally act to quickly initiate and resolve inflammation in response to PAMPs.[55] However,

in response to cellular death initiated by neutrophils macrophages have membrane processes in order to prevent the additional cytokine storm that would be induced in response to the aforementioned cell death. This method of "cloaking" debris from neutrophils and other inflammatory reactive factors dampens the inflammatory response so the resolution phase can begin.[54,55] Through this simplified mechanism we can understand that when the harmful inflammatory stimulus is present for extended periods of time or there is a disruption of the resolution of acute inflammation as mentioned previously, neutrophils could continuously release these cytokines thereby overwhelming the macrophage response.[56] This represents just one interaction in the complex environment of the inflammatory environment but dysregulation of even one of these pathways can propagate inflammation to the chronic level.

Inflammation has a so called "sweet spot" where pathogens or damage are handled in an ideal manner. In the case of chronic inflammation the inflammatory process continues until there is significant tissue damage or poorly structured tissue remodeling.[33-35] Often, in these conditions the overexpression of pro-inflammatory signals results in the dampening of "turn off" signals that should bring the inflammatory response back to homeostasis.[13] For example,

over expression of Lamin A/C in obese rats results in dysregulation of the Nf-κB pathway, preventing it from shutting down correctly.[36] As previously stated, this pathway results in the production of pro-inflammatory cytokines. Thus preventing this pathway from being degraded or slowed results in the release of chronic inflammatory mediators that go on to continuously signal for a pro-inflammatory environment. Similarly, the MAPK and IL-6/JAK/STAT pathways are common inflammatory pathways that when dysregulated can cause the switch to chronic inflammation or failure of inflammation resolution.[37-39] The MAPK pathway can be activated by an assortment of ligands including mitogens, characteristic pieces of pathogens (i.e. PAMPs) such as lipopolysaccharides (LPS), or stress signals.[39,42,43] This pathway involves phosphorylation of ERK1 and subsequent phosphorylation of ERK2 in the mitogen response or MAPK3 which then phosphorylates MAPK2 which phosphorylates MAPK1 in the stress response to activate cellular proliferation and differentiation.[44,45] However, these two pathways are much more readily turned on and off as they rely on phosphorylation of targets in order to further potentiate the signal, whereas Nf-κB is constitutively active in many immune cells.[38-41] This goes to show the further complexity of inflammation and the difficulties in addressing chronic inflammation.[79]

Acute Inflammation

Chronic inflammation is a complex process that can result from exacerbation of the acute inflammatory pathways. Acute inflammation is similarly complex but better understood and has therapeutic interventions that have been studied rigorously. Acute inflammation is generally a host protective mechanism that works to minimize damage to affected tissue, clear the pathogenic stimulus, and prevent further infection.[79] The innate immune system is the primary contributor to the generation of an acute inflammatory response.[9,57] As discussed previously, inflammatory signals such as PAMPs and DAMPS are spread throughout the affected tissue. These signals are then recognized by antigen presenting cells (APCs) such as dendritic cells (DCs), macrophages, and B cells through their cell surface PRRs and antigen receptors. Each type of APC can have a host of functions in the ensuing cascade. DCs have shown more potent activity in their role as APCs than the others. In addition, they are capable of stimulating T cell maturation and recruitment *in vitro* and *in vivo* through neutrophilic action.[59-61] This is an important intersection of the innate and adaptive immune responses that leads to healing.

Activated lymphocytes are responsible for most pathogen removal in the acute inflammatory response.[129] These lymphocytes include natural killer (NK) cells, T cells, and B cells. Activation involves remodeling of the cell and presentation of new surface receptors. An important marker of immune response to infection and further inflammation is the neutrophil to lymphocyte ratio (NLR).[62] This provides to clinicians essential information whether there is a healthy interaction between the innate and adaptive systems. Once activated in secondary lymphoid tissue lymphocytes can travel to the site of injury along chemotactic gradients. While lymphocytes are an important aspect of adaptive immunity they too have complex roles that can aid in resolution or cause further injury. It has been shown that inhibition of key T cell cytokine IL-17A alleviated organ disfunction after acute myocardial infarction.[63] However, there are also numerous studies where reduction or exhaustion of mature T cells resulted in negative outcomes for patients. COVID-19 and AIDS are two diseases that present with altered T cell counts and amelioration of symptoms is associated with increased functional T cells.[64,65]

Anti-Inflammatories

As more research was performed on the inflammatory pathways and their signaling patterns, targets emerged for consideration in the amelioration of inflammation. The most common form of anti-inflammatory drugs is the class of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDS act on the COX pathway inhibiting it from producing the pro-inflammatory PGEs specifically PGE_2.[66] Due to the vast array of action inherent to the COX pathway and the many cytokines it generates NSAIDs act not only as anti-inflammatories but also as anti-analgesics and antipyretics.[67] Many NSAIDs do not differentiate between the two isoforms of the COX enzyme, COX1 and COX2. COX1 has a protective function in the gastric mucosa as well as being a marker for sperm health in males.[67,68] COX1's protection of the gastric mucosa is also why chronic ingestion of NSAIDs is not recommended by physicians as they can cause GI disorders such as ulcers in the stomach and intestines.[69] COX2 is highly expressed at sites of inflammation. Its production of prostaglandins, which initiate many other pro-inflammatory mechanisms, promotes the inflammatory process and so naturally is the main target for NSAIDs. Aspirin, one of the most commonly employed NSAIDs, does not cause any GI disturbances and does not effect prostaglandin levels in the gastric lining. This is because aspirin specifically acts directly on COX2 but not COX1. Following this discovery researchers focused on COX2

specific inhibitors in order to avoid the damage caused by traditional NSAIDS. This is not to say that COX1 does not control inflammation however as studies in COX1 and COX2 knockout mice have shown that inflammation initiation by these enzymes may be site specific and dependent on the inflammatory stimulus.[29,69] COX2 inhibitors have shown promise as potent anti-inflammatories and have been approved for treatment but are still shown to increase the likelihood of GI bleeds and cardiovascular events.[70]

Corticosteroids are the other option physicians have for the repression of inflammation. Corticosteroids are routinely used in severe cases of inflammation and are the most potent exogenous inhibitors. The drawback for this class of drugs is the severe side effects associated with them.[71] As is inherent of steroids in general, these drugs act systemically and therefore are associated with numerous side effects in their treatment of particular pathologies.[72] Thus, it has become critical for researchers to find new classes of drugs that can mitigate the side effects associated with both NSAIDs and steroid therapies. This is where the research of Prof. Charles Serhan and his laboratory becomes a pivotal point in the resolution of inflammation.[73,79]

Resolution as an Active Process

Up until the early 2000's inflammation was thought to be a process that simply involved initiation and then a dependence on the body to passively clear inflammatory signaling to return to homeostasis.[130] However, there is now an appreciation for the return to homeostasis after inflammation being an active process mediated by lipid autocoid mediators termed "specialized pro-resolving mediators" (SPMs) discovered by Dr. Serhan.[73,75] SPMs are synthesized from omega-3 polyunsaturated fatty acids (PUFAs). The main PUFA that all SPMs are metabolites of is omega-3 fatty acids (DHA or EPA: resolvins, maresins, and protectins) or arachidonic acid (lipoxins). The SPMs that have been described so far include resolvins (Rv), Lipoxins (LX), maresins (MAR), and protectins.[77,81] SPM production is initiated by lipoxygenases, cytochrome P450 monooxygenases (CYP450s) and COX enzymes and therefore was first discovered in response to Aspirin treatment.[136] The production cascade is specific for each class of SPM and can be further differentiated by the separate derivates of each class. Resolvins, the first SPM to be characterized, include D-series (RvD) and E-series (RvE) derivatives that act on distinct pathways and have differing physiologic effects.[73] These effects are receptor dependent. The RvD class of resolvins is generated from docosahexaenoic acid (DHA) while RvE mediators are generated

from eicosapentaenoic acid (EPA).[74-78] Further synthesis pathways are described in (Figure 1) below.

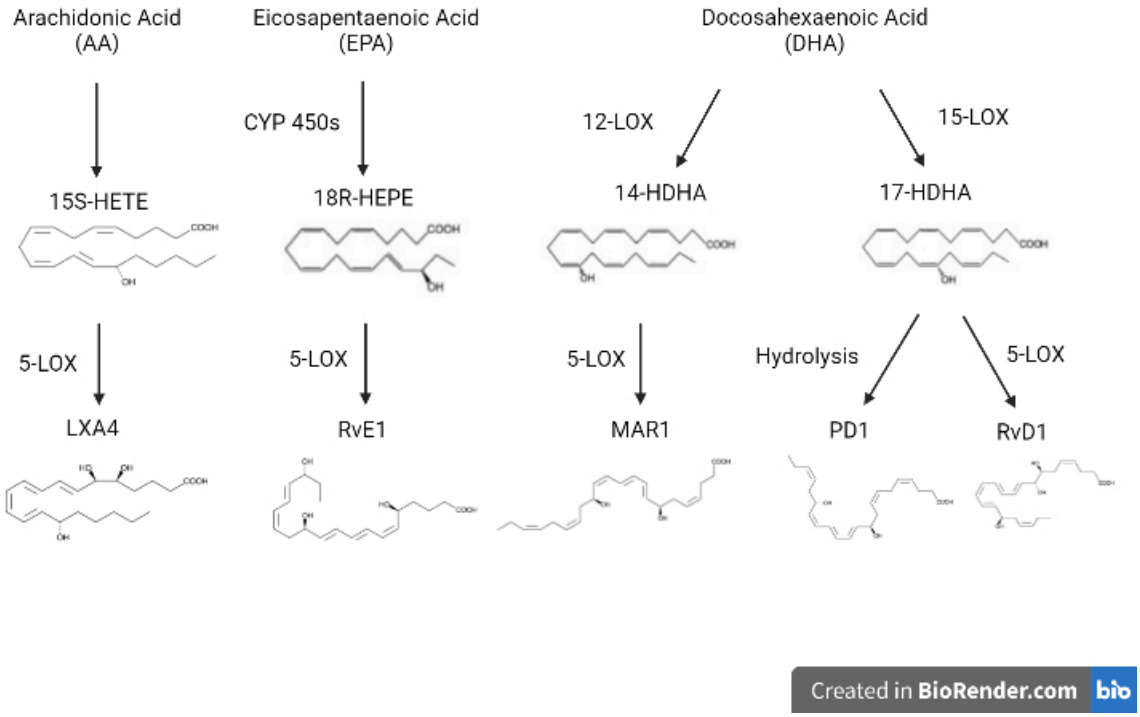

Figure 1. Synthesis of SPMs through the Arachidonic Acid, DHA and EPA Pathways

Arachidonic acid (AA) is the starting substrate for only one class of SPMs. In the case of maresins, resolvins, and protectins EPA or DHA is metabolized to through lipoxygenase (LOX) action. Further metabolization by CYP enzymes or lipoxygenases then yield the final lipid autocoid mediator products. Lipoxins are the only class of SPM produced from arachidonic acid without a DHA or EPA intermediate.

SPMs have diverse functions that are both tissue dependent and pathogen dependent. However, SMPs stimulate the resolution of inflammation through inhibition of neutrophil migration, increased phagocytosis by macrophages, and mitigating the effects of pro-inflammatory cytokines.[77,79] Studies on lipoxins

have elucidated their function in the normal inflammatory process. Neutrophils that recognize PGE_2 then cause a class switching of 15-LOX activity to produce lipoxins. This class switching results in increased macrophage efferocytosis and stop signals for neutrophil infiltration.[73,80,81] The distinction in action between the families of SPMs may arise from their separate surface receptor targets. Lipoxins utilize the ALX/FPR2 receptor to induce their pro resolving activity. Of note, this receptor can also function in a pro-inflammatory cascade which may further show why the expression of lipoxins leads to the resolution of inflammation in addition to its previously described functions.[77,82,118] RvD1 also utilizes the ALX/FPR2 receptor but can additionally bind the G protein coupled receptor (GPCR) GPR 32. RvD2 enacts its physiologic effect through the GPCR GPR18.[131] The E series resolving RvE1 utilizes the ChemR23 receptor.[118] In mice models, resolvins have been shown to play a similar role in the resolution of inflammation by regulating the function of macrophages and counter-regulating TNF-α induced pro-inflammatory cytokine levels.[83-85] Resolvins have also shown inflammation resolution activity in preventing the cytokine storm in Covid-19 through the regulation of prostaglandins and leukotrienes.[86] Protectins are primarily found in the brain and were first classified as neuroprotectins. The production of PD1 is done in a LOX-dependent manner as this enzyme is the

only known molecule capable of synthesizing it in peripheral blood mononuclear cells (PBMCs) and Th2 CD4+ T cells. While production has been discovered in these cells, the mediators themselves have been found in human microglial cells, peripheral blood and mice brain cells.[87,88] Protectins in particular can have differing functions based on modifications made to their structure. A common form of protectins that is inspiring new research is the sulfido conjugates. These conjugates play a role in tissue regeneration after injury and therefore are designated as protectin conjugates in tissue repair (PCTR). Synthetic protectins and PCTRs have similar roles in the resolution of inflammation and in disease models. As a class of SPMs, studies have shown their efficacy in limiting neutrophil infiltration, stimulating macrophage efferocytosis and phagocytosis.[89,91] PD1's anti-inflammatory and pro-resolving function also arises from inhibiting COX-2 and Nf-κB signaling. On the macroscopic level in human disease PD1 levels are associated with pathologies ranging from obesity, where protein expression is lower, to atherosclerosis that involves increased biosynthesis of PD1 in macrophages.[73,89-91] Maresins were the latest class of SPM to be discovered and described so the research into their function and role is limited compared to the other classes but rapidly expanding.[92] As previously stated, maresins are produced from DHA after being metabolized from free AA.

The major class of cells where these pro-resolving mediators are generated is the M2 class of macrophages. Briefly, macrophages are classed as either M1 or M2 depending on their function in the current microenvironment.[93] M1 describes macrophages that rely on a negative feedback controlled Th1 cell response to inhibit cell proliferation and cause tissue damage. M2 macrophages exert the exact opposite physiologic action promoting cell proliferation and tissue repair.[93] Maresins exert their physiological action by limiting neutrophil infiltration, class switching of macrophages, increased regulatory T cell (Treg) production, and inhibiting M1 macrophage differentiation.[95] In disease models such as acute lung injury maresins inhibit pro-inflammatory cytokine IL-6 production and restoring oxygenation.[91,94] Additionally, in both metabolic and vascular disease models maresins control macrophage actions such as M2 polarization, debris clearance, and inhibiting the release of pro-inflammatory signals TNF-α and IL-1β.[92,94,95]

SPMs in Clinical Trials

SPMs represent a paradigm shift in our understanding of the resolution process from a passive process to an active one.[73] Because they are endogenously produced, it is the goal of researchers and clinicians alike that the

druggability of these SPMs will represent a safer alternative to current anti-inflammatory and analgesic medications. Clinical trials are so far limited to administration of marine oils to increase the levels of endogenously produced SPMs but shown encouraging evidence to this end.[96] In 2020, a prospective study evaluated the efficacy of SPMs in increasing quality of life in patients with chronic pain for at least 3 months. Oral administration of marine oils normalized to resolvin and protectin precursor levels was evaluated over a 4 week time course. Result analysis showed significant beneficial changes in pain level and anxiety levels.[96] Another study involved adults with chronic inflammation being treated with EPA and DHA supplementation. After 10 week supplementation plasma levels of SPMs were altered with higher levels of EPA and DHA derivatives. Another important observation was the regulation of LPS-stimulated monocyte production of pro-inflammatory cytokines.[97] The major pro-inflammatory cytokines TNF-α, IL-6, MCP1 and IL-10; released from macrophages, were all decreased in response to DHA while only TNF-α was decreased by EPA.[97]

SPMs in Cancer

Because inflammation is a hallmark of cancer SPMs have emerged as a promising therapeutic option to limit tumor growth, metastasis, and comorbidities.[116] Chronic inflammation is known to exacerbate tumor promoting actions. This response can then lead to dormancy escape or recurrence of the tumor.[30] Current cancer therapeutics can act as a double-edged sword.[114] In order to halt tumor progression and rid the body of the cancerous mass clinicians often rely on cytotoxic chemotherapies or radiation that kills the cancerous cells in the area of the tumor. However recent studies suggest that this may promote tumor growth, metastasis and inflammation through the release of pro-inflammatory and cell death signals. This is known as the Révész effect.[98-101, 114] Therefore, it is evident that the issue of chronic inflammation in the tumor microenvironment must be resolved in order for the body to return to homeostasis.

The role of SPMs in the resolution of both acute and chronic inflammation influences signals known to enhance tumor progression. As has been discussed SPMs play a decisive role in controlling the Nf-κB pathway and activity levels of TNF-α. The cytokine TNF-α was first described as a protein that could cause necrosis of tumor cells hence its given name, and it had promise as a cancer treatment.[102] Unfortunately, it was found that it actually had very mild cytotoxic

effects and could actually inhibit the cytotoxic conditions that it was associated with in addition to having a plethora of harmful side effects.[102,103] The Nf-κB pathway is the strongest link between the chronic inflammatory environment and cancer. This pathway is involved in all hallmarks of cancer and therefore, Nf-κB is believed to play many roles in the tumor microenvironment including macrophage polarization, cytokine production, lymphocyte regulation, and cancer stem cell generation.[18,104-106] Many functions of Nf- κB are context dependent and can induce pro-tumorigenic and anti-tumor activity.

The urgent, unmet need for analysis of SPMs in inflammation resolution in the cancer setting is critical as they have potential as an adjuvant therapy for the gold standard treatments that can generate inflammation-induced tumorigenic activity.[116] Studies show that multiple SPMs are dysregulated in different types of cancer. In colon cancer it was discovered that RvD1 is inversely correlated with advanced stages along with an increase in TNF-α.[107] The lipoxygenase 15-LOX that is necessary for certain SPM synthesis is also downregulated in patients with colorectal cancer. In addition, aspirin which induces SPM formation reduces the risk of lung and breast cancers.[108,109]

SPMs are able to harness the pro-resolution activity of macrophages in cancerous tissues. This is vitally important in the context of cancer due to the

negative correlation between tumor associated macrophages (TAMs) and cancer prognosis. The complexity and plasticity of macrophages makes them viable targets for anti-cancer treatments but the context is important. M1 macrophages that are generally pro-inflammatory coincide with increased survival in non small cell lung cancer.[111] On the other hand, M2 macrophages that are generally anti-inflammatory and important in the resolution of inflammation are immune suppressive in cancer models preventing the necessary cytotoxic immune cells from migrating to the site and generating reactive oxygen species needed to kill the tumor cells.[110-111] Additionally TAMs are known to form tight interactions with cytotoxic CD8+ T cells.[113] This class of T cells is the body's most effective anti-cancer immune response as they target cancer cells through cell surface receptors and secrete cell-death inducing factors like granzymes and perforin.[112] Through macrophages and other routes of intervention SPMs induce their anti-tumor effect through mitigation of the inflammatory process and initiation of the resolution phase in cases of chronic inflammation.

sEH as a Target for Inflammation Resolution in Cancer

In the arachidonic acid pathway, CYP450 enzymes can generate the eicosanoids monohydroxyeicosatrienoic acids (HETEs) and epoxyeicosatrienoic

acids (EETs). The latter are known to have anti-inflammatory activity through suppression of the cytokine storm.[115] These EETs promote SPM production and are therefore beneficial in the resolution of inflammation. The enzyme soluble epoxide hydrolase (sEH) rapidly metabolizes these eicosanoids to the less active form dihydroxyeicosanoic acids (DHETs).[116,117] Our laboratory has previously shown that sEH inhibition, when combined with a COX2 inhibitor decreases tumor growth and prevents a macrophage dependent debris-stimulated cytokine storm in an ovarian cancer model.[115] sEH has also been shown to affect macrophage plasticity and its inhibition results in production of SPMs by resolving macrophages.[117] In order to prevent the cytokine storm associated with chronic inflammation and improve macrophage class switching, consistent with tumor regression, we utilize sEH inhibition in our studies to stimulate the resolution of inflammation.

AIMS AND GOALS

Aim 1:

- Show that resolution of inflammation in the TME inhibits tumorigenesis in multiple cancer models
- Evaluate the efficacy of RvE1 to inhibit tumor growth, burden, and number of tumor cells in the TME in a murine pancreatic cancer model

- Confirm the inhibitory actions of current frontline cancer treatments on tumorigenesis
- Demonstrate RvE1 is non-toxic and safe to implement as a novel adjuvant therapy to current cancer treatments.

Aim 2:

- Analyze activity of RvE1 on modulation of different immune cell types in the TME of murine pancreatic cancer models
- Demonstrate that RvE1 improves frontline treatment action in suppressing tumor growth, burden, and cell number
- Determine mechanism of action of RvE1 in exhibiting anti-tumor activity (i.e. receptor dependency)

Aim 3:

- Evaluate the anti-tumor activity of RvE1 on metastasis and tumor burden inhibition in murine metastatic cancer models
- RvE1 mechanism of action in metastasis inhibition through immune cell type analysis
- Associate RvE1 treatment with reduced metastases to lymph nodes

METHODS

Immunohistochemical Analysis:

Tumors of sacrificed mice were surgically removed and placed into 4% paraformaldehyde. Twenty-four hours later tumors were moved to 70% ethanol before transport to the histology core. At the histology core, tumors were embedded in paraffin. We then cut the paraffin blocks into 5-10μm thick sections and placed on glass slides. To prepare samples they were deparaffinized in xylenes 3x5minutes. Then rehydrated in 50%, 70%, 90%, 95%, and pure ethanol 5 minutes each. Endogenous ligand blocking was done with a 12 minute treatment of 3% hydrogen peroxide. Antigen retrieval using .1% sodium citrate involved immersing the slides, heating to a boil, and maintaining temperature just below boiling for 15-20 minutes. Next, another blocking step was performed using 3% bovine serum albumin in phosphate buffered saline. Primary antibody to murine Ki67 (Abcam cat:ab15580) was applied to slides and left overnight in our 4 degree Celsius cold room. On the second day secondary antibodies conjugated with biotin were applied for 30 minutes. Next streptavidin HRP was applied twice for 30 minute rounds with a 7 minute application of biotinylated tyramide between. Finally, sections were treated with the ImmPACT kit (Vector labs SK-

4105), Mayer's hematoxylin, and Tacha's bluing reagent. Times were ~1:30 minutes, 1 minute, and 15 seconds respectively. To seal the slides for visualization a drop of toluene was placed on slide with slide cover over it.

ImageJ Quantification:

Each mouse had their tumor removed and sectioned onto slides as described above. For each slide, 7-10 pictures were taken on a Leica Thunder microscope at 20x magnification that represented the staining of the entire tissue. Each image was then uploaded to ImageJ where they were first processed into RBG stacks to distinguish positive staining from counterstaining. Next, appropriate thresholds were used to evaluate positive staining. Thresholds were kept consistent unless brightness of the image impacted differentiation of positive and background staining. In which case, thresholds would be compared with the original image to get the most accurate representation of positive staining. Once threshold was established, parameters matching cell size and shape were implemented to further differentiate positive cells from background. Finally, the analyze function quantified the amount of positively stained cells which was then converted to number of cells/mm^3 based on the size of each image.

Mice:

6-8 week old C57BL/6 mice were purchased from Jackson Labs (Bar Harbor, ME). For subcutaneous studies, mice were shaved at least 24 hours prior to injection. Alcohol wipes were used to sterilize the area and 100μL of tumor cells suspended in PBS were injected into each mouse dorsally. Each mouse in the subcutaneous pancreatic cancer (KPCY) studies were injected with $3x10^5$ tumor cells. $5x10^5$ PANC02-H7 cells were injected for the orthotopic (directly into the pancreas), pancreatic cancer study. Mice were injected with either $1x10^6$ cells subcutaneously in PANC02-H7 primary tumor growth experiments or $1x10^4$ living PANC02-H7 cells and $9x10^5$ dead PANC02-H7 cells in knockout experiment. $1x10^6$ LLC tumor cells were injected subcutaneously for the metastatic LLC resection study. For metastasis analysis, we removed the left and right inguinal and axial lymph nodes after sacrifice, assessing each for metastasis. For subcutaneous studies mice were randomized after about one week or until tumors were visible, based on tumor volume. All tumor volume measurements were done by the same researcher using a standard caliper.

Statistical Analysis:

Statistical analysis for all tumor volume and survival studies was done on Graphpad Prism. Values were inserted into tables to create figures and significance was determined through a Student's T test or two-way Anova test. Significance was determined to be any P value under .05.

Osmotic Pump Implantation:

Mini osmotic pumps were loaded with 200μL of solution and released 15ng/day RvE1 or vehicle. Loaded pumps were placed in 50mL conical tubes with saline and kept in an incubator overnight. The next day, ChemR23 knockout and wildtype mice were kept anesthetized with isoflurane during the procedure through a small mask covering their nose and mouth. Pumps were then inserted into the peritoneum and sealed with sutures. Mice were monitored the following days for wound healing and general function.

RNA Extraction:

Tumor samples were prepped for bulk RNA sequencing analysis by RNA extraction. Snap frozen 20mg pieces of tumor tissue were homogenized with a RNeasy kit from Qiagen and centrifuged for 1 minute at 10,000g. Supernatant was removed and mixed with 70% ethanol, placed in a flow through column,

and centrifuged at same intervals. Flow through was then discarded. First buffer from kit then added to column and again centrifuged at same intervals. Flow through was discarded. Same process repeated with other two buffers from Qiagen kit. Column was centrifuged alone for 2 minutes at 16,100g to dry membrane. Finally, RNase free water was added to column and subsequently centrifuged for 2 minutes at 12,000g. RNA levels were confirmed by NanoDrop spectrophotometer. Samples were then sent to Novogene on dry ice for analysis.

RESULTS

There is a significant need to address chronic inflammation in the tumor microenvironment to suppress tumor growth and metastasis. We used supplementation or administration of SPMs to induce resolution of inflammation in the TME. We also wanted to evaluate the efficacy of resolution mediation in multiple tumor types to assess the individual influences. We performed histological assessment on subcutaneous KPCY tumors. KPCY is a murine model of human pancreatic ductal adenocarcinoma (PDAC) that closely resembles the biological effects of human pancreatic cancer.[119] We targeted Ki67, a tumor cell marker, during immunohistochemical analysis in order to evaluate the anti-

tumor activity of inflammation resolution on tumor burden in the TME. Additionally, mice were treated systemically with anti-PD1 (aPD1), an immune checkpoint inhibitor, that acts by destroying antibodies that target programmed cell death protein 1, preventing the proliferation of tumor cells that are unresponsive to normal cell cycle stop signals . Due to the immune suppressive environment of pancreatic cancer the efficacy of this drug is lower than in other tumor models . However, it is still used in combination with chemotherapy and invasive procedures such as surgery.[120,121] We evaluated the ability of SPMs to synergize with current treatments to bolster the immune defense through anti-inflammatory and pro-resolving mechanisms.

Mice treated with either aPD1 and/or our synthetic resolvin E1, a druggable RvE1 small molecule containing RvE1 molecules bound to magnesium L-lysinate, showed significant decreased tumor burden in paraffin embedded tumor tissues of KPCY. Visualization of sections (Fig. 2A) shows an obvious reduction in tumor cells between control and aPD1 monotherapy and combination therapy. ImageJ quantification (Fig. 2B) of the antibody-stained sections confirmed those results showing significant decreases in tumor burden.

Trending in the KPCY study also suggests that with a larger N, RvE1 as a monotherapy may be efficacious in reducing tumor burden.

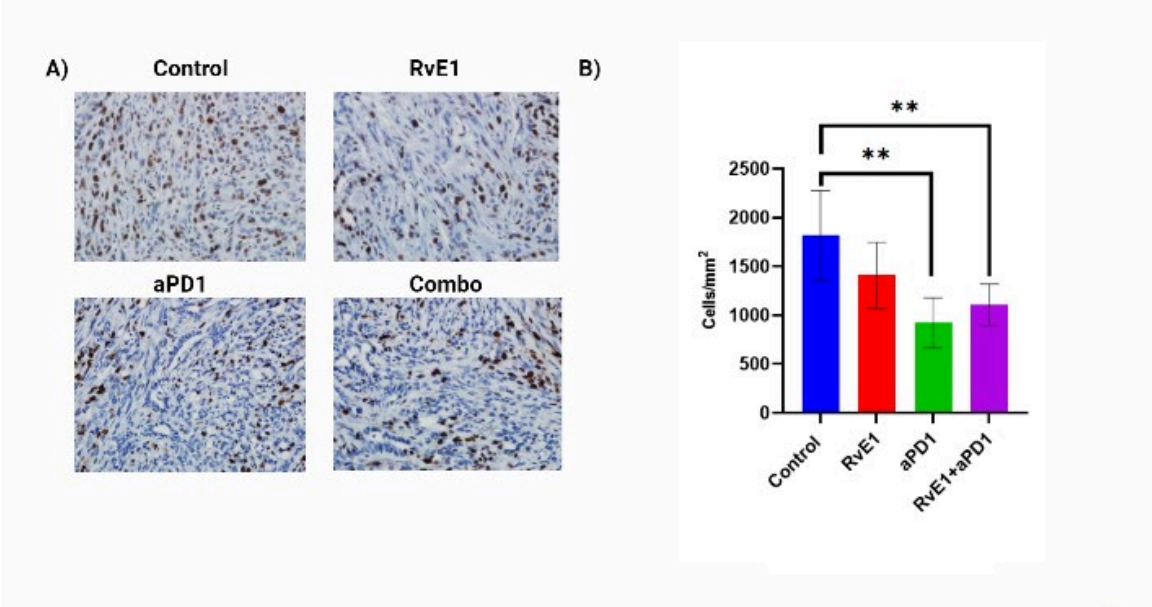

Figure 2. RvE1 Reduces Tumor Burden with aPD1 treatment

Four groups (N=10) of mice injected with 300k KPCY tumor cells subcutaneously were treated by intraperitoneal injection with either 58μg/ml RvE1 Q6 and/or 200μg/kg aPD1 Q3. Treatment began one week after injection to allow for tumor growth. Mice were treated for 20 days before sacrifice. A) 20x magnification of KPCY tumor tissue stained with primary antibody targeting Ki67 and a HRP conjugated secondary antibody. B) ImageJ quantification of diaminobenzidine chromogen labeled Ki67 antibody staining. 10 representative sections per mouse. ** P< .05 compared to control.

To confirm the efficacy of RvE1 in pancreatic cancer and the activity in a metastatic model we implemented studies with the PANC02-H7 (mouse pancreatic ductal adenocarcinoma metastatic model) cell line. Despite being the leading cause of cancer death (over 90% of cancer deaths), metastasis is an understudied aspect of cancer physiology.[133] To this end we evaluated the

ability of RvE1 to induce tumor suppressing activity in a metastatic murine model of PDAC. In addition, we simulated a highly inflammatory setting to induce metastasis by co-injection with dead PANC02-H7 tumor cells (Fig. 3B). This technique was inspired by our findings that dead cell debris stimulated tumor growth and metastasis.[100,114] Tumor volume was decreased in mice treated with both RvE1 and aPD1 monotherapies however the most drastic reduction in volume was observed in the combination group with a more than 75% difference in volume compared to placebo (Fig. 3A). Once we confirmed the efficacy of exogenous RvE1 treatment it was necessary to understand whether its action was receptor dependent. Studies in the metastatic PDAC model confirmed the action of RvE1 in suppressing tumor growth was receptor dependent. ChemR23 knockout mice receiving no treatment, ChemR23 knockout mice with RvE1 treatment, and wild type (WT) mice all showed increased tumor volume compared to wild type mice receiving RvE1 through a mini osmotic pump (Fig. 3B).

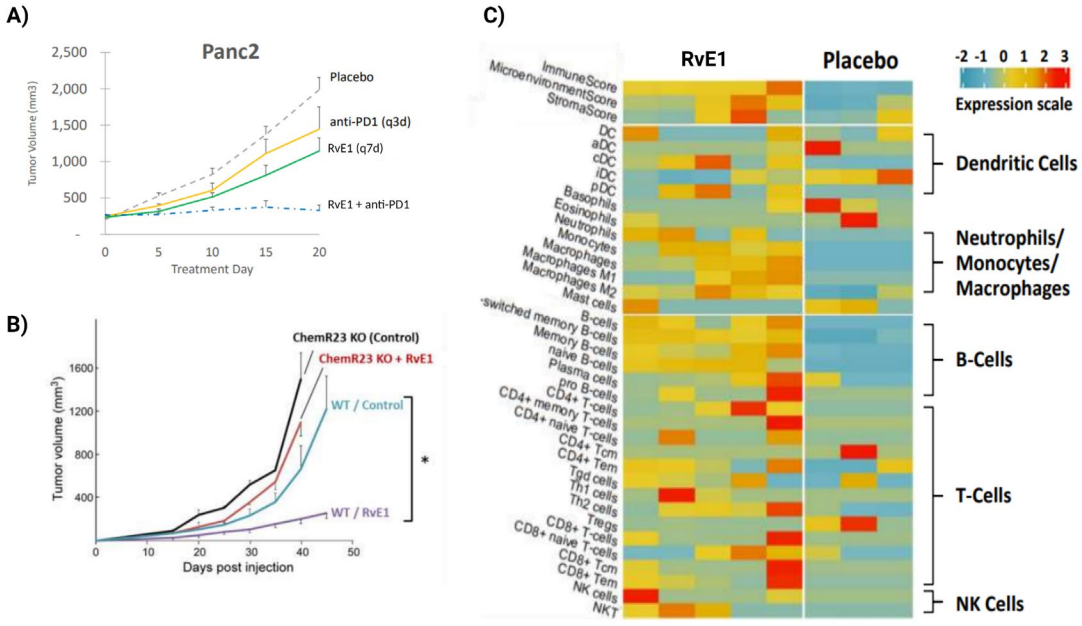

Figure 3. Tumor Growth is Associated with RvE1 Activity in a Metastatic PDAC Model

A) We injected C57BL/6 mice subcutaneously with 1x10^6 live PANC02-H7 cells and randomized into four groups (N=8). Treatment with placebo, RvE1 (30 ug/kg, SC, q7d), anti-PD1 (200 ug, IP, q3d) or RvE1 and anti-PD1 was initiated when tumors reached ~240 mm^3. B) ChemR23 KO or WT Subcutaneous injection with 1x10^4 Panc02-H7 living cells and 9x10^5 Panc02-H7 dead cells (N= 4–5 /group) and treated with RvE1 (15 ng/d) or vehicle via mini-osmotic IP pump beginning on Day 0.* P<0.05 compared to WT control. C) Heat map of immune cell profiles. SC injection of 1x10^6 living Panc02-H7 in C57BL/6J mice. Treatment with placebo or RvE1 (300 ug/kg, SC, q7d) initiated when tumors reached > 200 mm3. Tissue samples analyzed by bulk RNA-seq.

We performed bulk RNA seq to evaluate the mechanism of action of RvE1 in the PANC02 model (Fig. 3C). Consistent with previous studies and the mechanism of SPMs, RvE1 increased immune cell infiltration. The most notable effects were seen in neutrophils, monocytes, macrophages, and B cells.

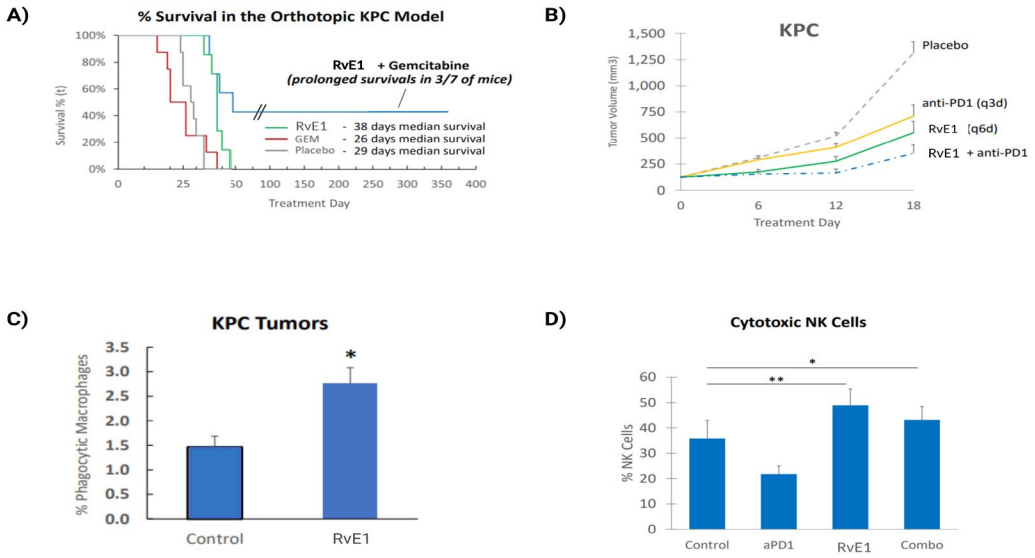

Figure 4. Neo-adjuvant RvE1 Therapy Improves Frontline Treatments and Influences Immune Cell Activity

A) Orthotopic injection of 5x10^5 KPC cells in C57BL/6J mice. Treatment with placebo, RvE1 through day 150 (300 ug/kg, SC, q6d), gemcitabine (50 mg/kg, q3dx6), or RvE1 and gemcitabine, initiated 6 days after implantation. n=7-8/group. B) SQ injection of 3x10^5 KPC cells in C57BL/6 mice. Treatment with placebo, RvE1 (300 ug/kg, SC, q6d), anti-PD1 (200 ug, IP, q3d) or RvE1 and anti-PD1 was initiated when tumors reached ~130 mm3. Combo vs anti-PD1 ($p < 0.05$); vs placebo ($p < 0.001$);RvE1 vs placebo ($p < 0.001$); anti-PD1 vs placebo ($p < 0.001$). N=10/group. C/D) Mice were injected subcutaneously with KPC tumor cells and treated with vehicle or RvE1 (SC, 300 ug/kg, Q6D). On day 18, mice were sacrificed, and tumor tissue was analyzed by flow cytometry. n = 4 mice/group. Markers: CD11b+ x F4/80+ x CD163+ x $CD206^+$ (Phagocytic macrophage), $CD3\varepsilon^-$ x NK.1.1 x $NKG2D^+$ (Cytotoxic NK cells)

SPMs can exhibit distinct characteristics in different cancer models. We set out to evaluate the mechanism of action of RvE1 in the KPCY model. To do this we implemented standard survival studies in an orthotopic model of pancreatic cancer. Orthotopic models are ideal in cancer studies as they more closely

represent the natural response of the body in the affected tissue.[134] Mice treated with RvE1 showed enhanced survival when compared to placebo treatment in the orthotopic model. More importantly, RvE1 showed synergy with another frontline cancer chemotherapy treatment, gemcitabine (Fig. 4A). The combination group induced prolonged survival in three out of the seven mice despite all other groups reaching a max survival time of just under 50 days. Remarkably, almost a year after the study began the combination treated mice are still living and healthy. We then confirmed our findings that RvE1 reduced tumor volume in the KPCY model (Fig. 4B). Both monotherapies had significant reductions in volume compared to placebo. The combination group was also significantly reduced compared to placebo, but interestingly was also significantly lower than both monotherapies showing that it did indeed synergize with aPD1 treatment. Flow cytometry analysis was then performed in the KPC model to analyze the mechanism of action in this model (Fig. 4C/D). Results showed RvE1 caused the reprogramming of macrophages to a phagocytic phenotype. This result was significantly increased compared to placebo. Furthermore, the anti-inflammatory and pro-resolving actions of RvE1 stimulated increased NK cell infiltration into the TME both as a monotherapy and in combination with immunotherapy (aPD1).

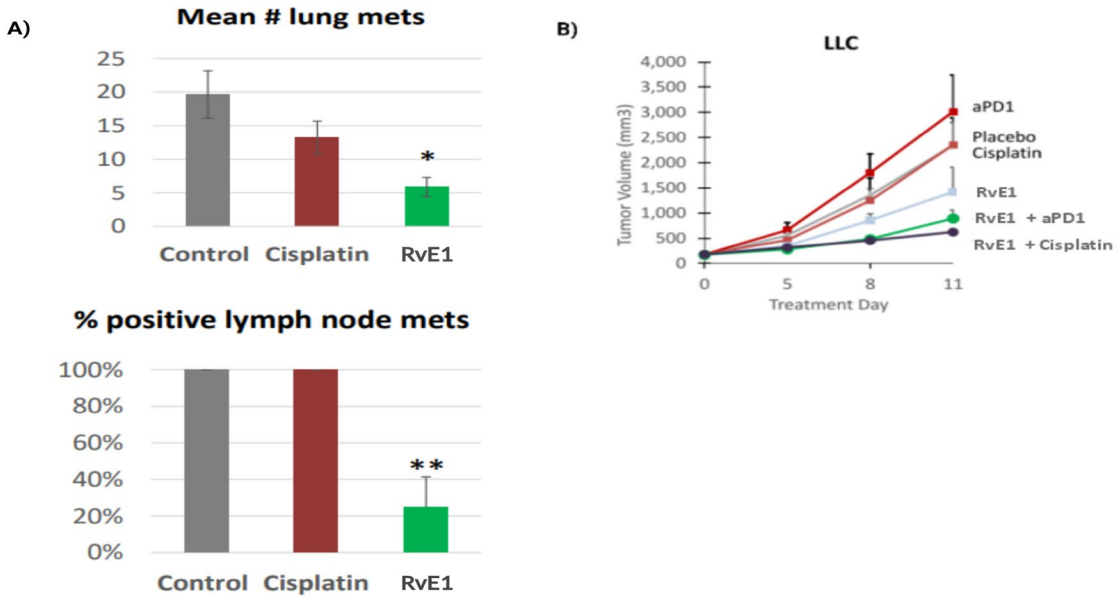

Figure 5. RvE1 has Anti-metastatic and Anti-tumor Activity in Lewis Lung Carcinoma Resection Model

A) Mice injected subcutaneously with 1x10^6 LLC tumor cells. Once tumors were ~1500mm^3, mice received placebo or RVE1 (SC, 300 ug/kg) (N=3-8/group) 2 hr before resection and on days 6, 12 and 18 after resection or Cisplatin (IP, 5 mg/kg) 24 hr before resection and on days 6 and 12 after resection. Mice were sacrificed on day 19 after resection. Lymph node mets were excised from left/right axillary and inguinal lymph nodes. B) SQ injection of 1x106 LLC cells in C57BL/6 mice. Treatment with placebo, RVE1 (30 ug/kg, SC, qd), cisplatin (5 mg/kg, IP, q5d) or RVE1 + cisplatin was initiated when tumors reached ~170 mm3. Combo vs placebo ($p < 0.05$); vs cisplatin ($p < 0.05$). (N=5/group)

Because SPMs have been demonstrated to exhibit pro-resolving functions through counter-regulation of cytokine storm mediation in other respiratory diseases such as Covid-19, SPMs would likely mitigate tumor activity in lung cancer through similar mechanisms. To test the efficacy of RvE1 in a lung cancer model and observe the influences on metastasis in another model we performed tumor growth studies in Lewis Lung Carcinoma (LLC). In our study we found

that the standard chemotherapy treatment, cisplatin, did not significantly reduce tumor volume or lymph node metastasis as a monotherapy. However, RvE1 exhibited significant reductions in both metastasis and tumor volume (Fig. 5A). RvE1 also inhibited tumor growth in a standard LLC subcutaneous model as a monotherapy and was able to synergize with two common lung cancer treatments in the immunotherapy aPD1 and chemotherapy cisplatin (Cis). Combination therapy with RvE1 and cisplatin improved anti-tumor activity with further reduction of primary tumor growth.

DISCUSSION

SPMs synergize with current frontline cancer treatments to control the immunosuppressive, pro-inflammatory TME. Immune suppression in the TME is enhanced by inflammation-associated tumor cell death ("debris"), which is generated from the cytotoxic effects of immune cells like NK and $CD8^+$ T cells. Stimulation of resolution of inflammation causes class switching of macrophages to phagocytose tumor-promoting debris which further limits the pro-inflammatory cytokine storm released in response to cell death. In our studies we demonstrated that the SPM RvE1 reduces tumor burden in the TME. This was determined through our histological assessment as there is a dramatic reduction

in tumor cell marker positive cells. RvE1 is known to activate its receptor ChemR23 on antigen presenting cells.[122] These APCs then stimulate resolution which includes activating macrophages to phagocytose debris and counter-regulate pro-inflammatory cytokine signaling as we demonstrated through our RNA sequencing analysis. Inhibition of this signaling leads to greater immune cell infiltration that can kill tumor cells resulting in a lower tumor burden confirmed by histochemical analysis and tumor volume analysis. Cytokine storms have been shown to be the mechanism of injury in many other diseases so mitigating this induction of signals is the bare minimum needed to achieve inflammation resolution mediated treatment in the TME.

The ability of RvE1 to induce NK cell infiltration and phagocytic macrophage generation in the TME represents an important finding for the specific cell types that RvE1 enacts its activity through. SPMs can exhibit multiple pleiotropic effects in multiple cancer types makes these findings relevant to many cancer types which are driven by inflammation and/or the loss of resolution.[30,116,121,135] There is now evidence that RvE1 acts in a similar manner in pancreatic cancer as has been shown in non-cancer models. Neutrophil infiltration, macrophage class switching, NK cell mediated damage mitigation are all known mechanisms in resolvin mediated inflammation resolution. [83-85]

NK cells act not only as cytotoxic cancer killing cells, but they also induce T cell responses that increase the cytotoxicity of the T cells.[128] Researchers have been testing exogenous NK cell injection to treat cancer. However, they do not elicit a long acting response and can be vulnerable to the many inhibitory signals active in the TME.[128] In our cancer models RvE1 increasing the percent of cytotoxic NK cells in the TME was indicative of overcoming the challenge represented by exogenous NK cell treatment. This may be through the suppression of T regulatory cells as demonstrated in bulk RNA seq that can suppress NK cell cytotoxicity. The other potential mechanism could be the activation of NK cells that then are less likely to be suppressed by Tregs. In addition, the increase of cytotoxic NK cells can induce memory T cells to remember cancerous cells and their markers. This could result in lower levels of recurrence and anti-metastatic effects systemically, although further experimentation would be required to prove these hypotheses.

The importance of ChemR23 became even more apparent in murine knockout studies. Tumor growth was significantly specifically inhibited in only WT mice treated with RvE1. The fact that ChemR23 knockout treated with RvE1 showed no inhibition of tumor growth demonstrates that the action of RvE1 is receptor dependent. This aligns with current knowledge on the anti-tumor action

of ChemR23. The leukocyte chemoattractant chimerin also uses ChemR23 as a receptor and exhibits anti-tumor activity.[123] With these findings it is apparent that ChemR23 is an important mediator of anti-tumor activity in the TME through its action in inflammation resolution as well as recruitment of cancer fighting leukocytes. It is also important to note that throughout our RvE1 studies no apparent toxicity was shown despite invasive treatments. This finding is likely because RvE1 is an endogenously produced molecule unlike current anti-inflammatory therapeutics such as NSAIDs. Stimulation of resolution of inflammation is an exciting novel approach that is very different from most frontline current cancer therapies (e.g. chemotherapy and radiation) which induce pro-tumorigenic inflammation or harmful systemic side effects on the body.

For the knockout study we utilized mini-osmotic pumps that provide a slow release of the desired lipid mediator into the peritoneum where it could be absorbed systemically. Pump administration allowed us to conclude that the mice were all receiving similar, continuous dosing at very low concentrations (nanogram/day) which is over 1000-fold lower than other anti-inflammatory drugs and chemotherapy. Additionally, without the need for human intervention during treatment, mice were not subjected to any added stress which is known to

negatively impact physiology and behavior.[124] This technique showed there are multiple effective routes of administration of SPMs including intraperitoneal, oral, and subcutaneous. However, there are currently no FDA approved SPM medications given through invasive administration routes.

Metastasis is associated with the worst prognosis in all cancer types. Therefore, it is critical to address anti-metastatic activity in cancer studies. We used the PANC02 as a model of metastatic pancreatic cancer. PANC02 bulk RNA sequencing data provided insight into the mechanism of lower tumor burden and immune cell effects. The increases in neutrophils and macrophages confirmed our understanding that anti-inflammatory and pro-resolving signaling mediated leukocyte infiltration to the TME. Studies have shown that while neutrophil and macrophage activity is important in fighting cancer they can also have pro-tumorigenic activity.[125] The most important takeaway from the bulk RNA seq data was not just the infiltration of leukocytes but that this action was mediated by SPMs due to their anti-inflammatory and pro-resolving properties. When combined with the anti-tumor activity shown in the *in vivo* models these results suggest the resolution of inflammation induces its anti-tumor activity directly in the TME by modulating the immunosuppressive defense mechanisms of cancer.

In addition to addressing metastasis in pancreatic cancer, a highly metastatic model would suggest that beneficial treatments could be applied to a wide range of cancers that metastasize. We evaluated resolvins (e.g. RvE1) in this LLC tumor resection model which is known to induce spontaneous lung metastasis after tumor resection. This model could also represent the efficacy of RvE1 as an anti-inflammatory given after surgery for tumor removal in humans. Any invasive procedure like surgery will generate inflammation as the body attempts to protect itself from further injury. If RvE1 shows efficacy in both cancer resection models and inflammation resolution it would be expected that it would improve outcomes in this demographic of cancer patients undergoing surgery, chemotherapy, and radiation. RvE1 therefore represents a promising novel approach to addressing cancer therapy-induced inflammation. Additionally, because it did not generate any toxicity in our studies whether given as a monotherapy or combination therapy the upside for clinical application is tremendous. Repeatedly, we showed that RvE1 synergized with other front line cancer treatments such as chemotherapy to improve their function while maintaining its function in stimulating resolution and influencing immune cells. Taken together these findings provide compelling evidence for clinical application of resolvins in multiple tumor models.

What we found extremely interesting in our metastasis models was that current clinical treatments minimally reduced tumor burden or number of metastases. With metastasis being the leading cause of death in cancer patients, the fact that these treatments showed a lack of efficacy in addressing this issue is problematic. However, with SPMs showing efficacy in tumor infiltration and inflammation resolution there may be a mechanism linking inflammation resolution and metastasis. It is known that the epithelial-mesenchymal transition of cancer cells converts their phenotype to one similar to a fibroblast. Indicative of this transition, tumor cells become much more motile and are able to escape from the primary location into the bloodstream.[126] Tumor cells that conform to this phenotype display stem cell like traits. EMT makes metastatic tumor cells more resistant to most treatments and more apt for recurrence . If this transition is influenced by the chronic inflammation in the TME then treatment with RvE1 may be preventing this phenotype switch of tumor cells. To prove this, we would need to test for markers of cancer stem cells and immune resistance in control and SPM treated groups. This represents an entirely novel approach to inhibit metastasis via the stimulation of resolution of inflammation.

CONCLUSION

There is an urgent need in the medical community in order to evaluate the role of endogenously produced pro-resolving lipid autocoid mediators in inflammation in the tumor microenvironment. SPMs have proved effective in promoting resolution and decreasing the cytokine storm that can result in continuous inflammation and tissue damage. We focused our efforts on the resolvin class in multiple models of pancreatic cancer and a metastatic LLC model. Our experiment results elucidated a novel adjuvant treatment option for early and late stage pancreatic cancer and other metastatic cancers. The issue of chronic inflammation is inherent to cancer and a host of other deadly diseases. However, there is currently no frontline treatment without serious side effects. Resolving inflammation in cancerous tissue and surrounding areas stimulates movement of cancer fighting cells to the affected area and inhibits the pro-tumor signals released in response to inflammation. RvE1 proved non-toxic in all studies and while maintaining its own mechanism of action it also perpetuated the effects of leading treatments used in the clinic today. This suggests that exogenous treatment with RvE1 in multiple tumor types may be safe and effective in mitigating metastasis and tumor growth in pancreatic cancer while increasing or maintaining the same effects of typical chemo and

immunotherapies. With such promising results in the RvE1 studies it will be important going forward to see the effects of the other SPMs in pancreatic cancer and metastatic models. Additionally, we want to see the results of RvE1 in other cancer models especially ones known to metastasize such as breast and bladder cancers. Finally, in relation to our current studies it will be important to elucidate the exact interaction of the SPMs with immune cells such as T lymphocytes, and NK, dendritic, and macrophage cells. Exact signaling pathways could give new targets for combination therapies in conjunction with SPMs and the standard chemo/immune therapies.

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