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IL-33 biology in cancer: An update and future perspectives

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ABSTRACT

Interleukin-33 (IL-33) is a member of the IL-1 family of cytokines that is constitutively expressed in the nucleus of epithelial, endothelial and fibroblast-like cells. Upon cell stress, damage or necrosis, IL-33 is released into the cytoplasm to exert its prime role as an alarmin by binding to its specific receptor moiety, ST2. IL-33 exhibits pleiotropic function in inflammatory diseases and particularly in cancer. IL-33 may play a dual role as both a pro-tumorigenic and anti-tumorigenic cytokine, dependent on tumor and cellular context, expression levels, bioactivity and the nature of the inflammatory environment. In this review, we discuss the differential contribution of IL-33 to malignant or inflammatory conditions, its multifaceted effects on the tumor microenvironment, while providing possible explanations for the discrepant findings described in the literature. Additionally, we examine the emerging and divergent functions of IL-33 in the nucleus, and aspects of IL-33 biology that are currently under-addressed.

1. Introduction

The IL-33 protein was first endogenously visualized as a nuclear protein highly concentrated in high endothelial venules (HEVs) [1]. It was eventually categorized into the IL-1 family of cytokines due to structural similarities and binding to a heterodimer receptor constituted by IL1RAcP (encoded by *IL1RAP*) and ST2 (encoded by *IL1RL1*, and representing the IL-33-specific moiety of the receptor), which belong to the IL-1 family of receptors [2,3]. Since then, the structure of the IL-33 protein has been extensively studied, mainly in humans and mice, with identification of its receptor binding region and multiple cleavage sites by serine/cysteine proteases [4,5]. Extracellular inflammatory proteases released by immune cells recognize multiple cleavage sites of human IL-33, mainly in its central domain, whereas intracellular proteases cleave its IL1-like cytokine domain [5]. These cleavages lead to shortened isoforms of IL-33 that display different efficacies to engage the signaling downstream of the IL-33 receptor (IL-33R). The precursor form of IL-33 is generally termed full length IL-33 (flIL-33), while processed isoforms of IL-33 is referred to mature IL-33 (mIL-33) [5]. ST2 transduces the signal triggered by its ligand via the MyD88/IRAK4 signaling complex, which leads to the downstream activation of various transcription factors such as NF-κB [6].

Expression of both IL-33 and ST2 can be inducible on a wide variety of cells from both hematopoietic and mesenchymal origins. However, during steady-state, IL-33 is mainly found in barrier cells (epithelium, endothelium, fibroblasts), whereas ST2 is mainly expressed on a large variety of immune cells [5,6].

The current paradigm regarding IL-33 biology states that it localizes to the nucleus due to a signal sequence in the N-terminal, from which it is released extracellularly upon cell injury, stress or necrosis. Thus, IL-33 was categorized as an alarmin similar to IL-1α, where its extracellular activity depends on binding to its receptor. Similar to other IL-1 family cytokines, IL-33 is mainly expressed in a precursor form that can undergo post-transcriptional cleavages for modification of bioactivity, as

Abbreviations: AML, acute myeloid leukemia; AOM, azoxymethane; CLL, chronic lymphocytic leukemia; COPD, chronic obstructive pulmonary disease; CRC, colorectal cancer; DSS, dextran sodium sulfate; EMT, epithelial-to-mesenchymal transition; endoMT, endothelial-to-mesenchymal transition; ESC, epithelial stem cell; flIL-33, full-length IL-33; GLUT1, glucose transporter 1; HDM, house dust mite; HEV, high endothelial venules; HNSCC, head and neck squamous cell cancer; HPV, human papilloma virus; IL, interleukin; ILC2, innate lymphoid cell type 2; MDSC, myeloid-derived suppressor cell; MerTK, c-Mer protooncogene tyrosine kinase; NK, natual killer cell; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; PPARγ, peroxisome proliferator-activated receptor gamma; sST2, soluble form of ST2; ST2, suppressor of tumorigenicity; TAM, tumor-associated macrophage; TCGA, The Cancer Genome Atlas; TE, transposable elements; Th, T helper; TME, tumor microenvironment; Tregs, regulatory T cells; USP, ubiquitin-specific protease.

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indicated above [5]. It is important to note that both flIL-33 and mIL-33 are bioactive and physiologically relevant during inflammation and disease, albeit at different potencies and with the latter having higher bioactivity [5,7,8]. In general, as IL-33 lacks a secretion sequence [2], release of either flIL-33 or mIL-33 is thought to be passive, via damaged plasma membranes of necrotic cells. Extracellular IL-33 can be further activated through cleavage by immune-cell derived proteases in the extracellular environment [5,9,10].

To prevent inappropriate inflammation due to unsolicited IL-33 in the milieu, nuclear sequestration of IL-33 serves as an important homeostatic sink to restrain uncontrolled extracellular release of this alarmin [11]. Furthermore, IL-33 can be intracellularly inactivated by proteases during apoptosis and can also be extracellularly suppressed via cysteine oxidation [5,12]. Therefore, IL-33 activity is spatiotemporally restricted both by apoptosis-induced cell death, and also by various suppressive processes in the extracellular compartment. Since the establishment of this paradigm of the alarmin function of IL-33, there has been several new findings that question the simplicity of this model, which we discuss in this review.

Physiologically, IL-33 has been mainly implicated in different disease contexts and cancer types [13–18]. Binding of IL-33 to its receptor on immune cells generally results in activation/proliferation of different cell types and orchestration of a type 2 immune response. However, IL-33 may also stimulate immune cells associated with type 1 immune responses, such as natural killer (NK) cells, $CD4^+$ T helper 1 (Th1) cells, and $CD8^+$ T cells [19–21], which illustrates the pleiotropic nature of this cytokine. Indeed, IL-33 is able to induce anti-inflammatory and pro-repair effects, via modulating $CD4^+$ regulatory T cell (Treg) function and production of amphiregulin [22–24]. As such, IL-33 is now recognized as playing a critical role for the regulation of innate and adaptive immunity through its regulation of tissue homeostasis and other pathological conditions, such as allergy, infection and cancer [25]. IL-33 has been shown to drive disease pathology or to promote resolution of inflammation during different inflammatory conditions and diseases [15,20,26–28], yet there are now multiple studies, in particular in the field of cancer, that indicate contradictory functions of IL-33 and IL-33-induced signaling.

While IL-33 biology and its role in inflammatory diseases or cancer have been extensively well reviewed in the past years (5,25,29–31], here we aim to provide updates on aspects of IL-33 biology that are still ambiguous. In particular, we aim to present and propose possible clues for the discrepancies in the role of IL-33 in certain cancers, as well as discuss potential mechanisms that might explain these contradictions. We will review the regulation of IL-33 expression and bioactivity in different disease contexts and extrapolate these findings to relevant aspects of tumorigenesis or immunoregulation in cancer. Moreover, we also discuss a possible emerging nuclear role of IL-33 that might add to the complexity of IL-33 function and highlight a need to reinterpret our current knowledge of IL-33.

2. Contradictory roles of IL-33 in tumorigenesis

For certain cancer entities, the precise function of IL-33 remains unclear, with divergent reports on the role of this cytokine in the tumor microenvironment, depending on the type of cells expressing the IL-33 or IL-33R, the species studied, or the experimental model and readouts applied [31]. Here, we focus on reviewing the role of IL-33 in the most investigated malignancies with contradictory findings on IL-33 function, which we illustrate with representative reports from the literature. Further studies are referred to in Table 1.

2.1. Role of IL-33/ST2 in lung cancers

Lung cancer is the most commonly diagnosed cancer and remains a worldwide health problem, with a high yearly death toll [32]. Between 80 and 85% of patients with lung cancer are diagnosed with non-small cell lung cancer (NSCLC) [32]. Although many studies have shown that IL-33/ST2 signaling plays a role in inflammatory lung diseases, such as chronic obstructive pulmonary disease (COPD), respiratory allergy and asthma [25,33], few have investigated how this pathway contributes to lung cancer. IL-33 levels were found to be higher in sera of patients with NSCLC compared to healthy donors, suggesting an involvement of this cytokine in regulating this type of tumor [35].

Some studies found that the IL-33/ST2 axis has pro-tumorigenic effects on cancer cells. Stimulation with IL-33 in *in vitro* and *in vivo* models of NSCLC led to outgrowth and metastasis by enhancing the membrane protein glucose transporter 1 (GLUT1), thus increasing glucose uptake and glycolysis [35], a characteristic of the Warburg effect that is central for the growth of tumor cells [34]. Moreover, metastasis and growth of NSCLC tumor cells were enhanced upon bacteria-induced increased

Table 1
Interleukin-33 (IL-33) and ST2 levels and contribution in different cancers.

Type of cancer		Net effect of IL-33: Tumor progression/cell growth	IL-33: Metastasis	ST2: Tumor progression/cell growth	ST2: Metastasis	References
Lung	Human	–		-		[37] Akimoto et al. 2016
		+	+	+	+	[35] Wang et al. 2016
		-				[38] Kim et al. 2015
	Cell	+	+			[36] Sun et al. 2018
Breast	Mouse	–	–	–	-	[43] Gao et al. 2015
	Cells	+				[42] Jovanovic et al. 2014
		+	+			[38,40] Kim et al. 2015, Kim at al. 2021
Colon	Human	+	+	+	+	[202] Liu et al. 2014
		+	+			[44] Mertz et al. 2015
	Mouse	+	+	+		[47] Maywald et al. 2015
		+	+	+		[44] Mertz et al. 2015
		–	-			[53] Malik et al. 2016
	Cells	+	+	+	+	[202] Liu et al. 2014
Myeloproliferative	Human	+				[61] Mager et al. 2015
neoplasms	Mouse	+		+		[61] Mager et al. 2015
Acute myeloid leukemia	Mouse	–	-			[65] Qin et al. 2016
Gastric	Human	+	+	+	+	[64] Yu et al. 2015
Head and neck squamous	Cells	+	+			[54,55] Chen et al. 2013, Ishikawa et al. 2014

+, promoting effect; -, suppressing effect

Interleukin-33 (IL-33) and ST2 levels and contribution in different cancers.

expression of *IL33* mRNA and IL-33 protein [36].

However, other reports have suggested protective effects of the IL-33/ST2 axis on halting progression of lung cancer, indicating an inverse correlation between IL-33 levels and stages of human lung cancer and overall survival [37,38]. In accordance with these findings, ST2 was expressed mainly in low-metastatic, but not high-metastatic, cells in murine Lewis lung carcinoma cells [37]. Overall, while mechanistically IL-33 seems to support tumor growth, findings on IL-33/ST2 expression in different cancer stages or metastatic activity do not support this notion, thus indicating the need for further research in this area.

2.2. Role of IL-33/ST2 in breast cancer

Breast cancer is a major cause of cancer-related deaths among women [39]. Although standardized multi-modal treatment and technologies have greatly improved overall outcome and quality of life for breast cancer patients, new therapeutic targets and prognostic markers are needed. Several studies report a potential contribution of IL-33 to breast cancer. In patients, levels of IL-33 or ST2 protein expression were increased in cancer lesions compared to matched normal breast tissues [39,40].

In experimental studies, IL-33 showed a direct pro-tumorigenic action by enhancing proliferation and colony formation of breast cancer cell lines [40,41]. In a 4T1 breast cancer mouse model, mRNA and protein expression of *Il33* were upregulated in primary tumors as the cancer progressed [42]. Together, these different findings indicate that IL-33 acts as a pro-tumorigenic cytokine that favors tumorigenesis and down-modulation of anti-tumor immunity. Despite these findings, two separate studies using the same 4T1 breast cancer model showed that 4T1 cells engineered to overexpress IL-33 inhibited tumor growth and metastasis in mice [43].

In summary, these conflicting findings on the contribution of IL-33 to tumorigenesis in the same 4T1 breast cancer model suggest an underlying mechanism of IL-33 activity that remains to be addressed.

2.3. Role of IL-33/ST2 in colorectal cancer

Despite substantial improvements in diagnosis and treatment over the last few decades, colorectal cancer (CRC) remains one of the most frequent cancer types with a potentially lethal outcome [44]. Evidence from clinical and experimental findings suggests that chronic inflammation promotes intestinal tumorigenesis, which is supported by the observation that patients with irritable bowel disease have a higher risk in developing CRC [45]. Multiple studies on CRC in human patients show a change in IL-33 or ST2 expression in tumor tissues, when compared to either adjacent normal tissues or healthy patients, which support the notion that the IL-33/ST2 axis contributes to intestinal tumorigenesis [44,46,47].

Our group previously reported an increase in IL-33 and ST2 protein expression in adenoma and low-grade human CRC, compared to adjacent normal tissue and more advanced adenocarcinoma [44]. In line with our findings, an independent study showed that stimulation of human primary CRC cells with exogenous IL-33 promoted growth, metastasis and invasion *in vitro*, while markedly reducing the survival time of immunosuppressed mice challenged with these cells [44,47,48]. Moreover, IL-33 promotes tumorigenesis in an $Apc^{Min/+}$ murine model of CRC, where IL-33 activity was associated with proteases and cytokines known to promote polyposis, and genetic ablation of *St2* protected tumor development in a model of chemically-induced CRC by reducing the frequency of $ST2^{+}$ Treg in tumor lesions [44,48].

Another cog in the pathogenesis of CRC is a variant isoform of ST2 termed soluble ST2 (sST2) that mainly acts as a decoy receptor to dampen IL-33 cytokine activity via sequestration [49] (see also the section on ST2 at the end of this review). An inverse association between sST2 expression and malignant cell growth has been reported, with a downregulation of sST2 in highly metastatic cells. In addition, CRC-derived sST2 suppresses the growth, metastasis and angiogenesis of the tumor, affecting both the stromal and immune compartment in the tumor microenvironment (TME), most likely by blocking IL-33/ST2 signaling in different cell types [50,51], thus highlighting a possible pro-tumorigenic role of IL-33 in CRC.

In contrast with these findings, other studies rather indicate a protective, anti-tumoral role of IL-33/ST2 signaling in CRC. In human CRC patients, expression of ST2L protein (the receptor isoform of *ST2* mediating intracellular signaling) was significantly decreased in tumor tissues compared to adjacent healthy tissues, with lower expression along tumor grades. Moreover, CT26 CRC cells with *ST2* knockdown showed enhanced growth in mice after engraftment, further suggesting an anti-tumoral role of ST2 under certain conditions [52]. *Il33*-deficient mice were more susceptible to chemically-induced CRC compared to their wild-type counterparts, showing an increase in tumor size, number and grade. Further analysis indicated a role of IL-33 for the maintenance of gut microbial homeostasis via the production of IgA in B cell, which was necessary to control intestinal inflammation [53].

While correlative data from patient studies indicate a possible involvement of IL-33 in CRC, they provide limited information on the underpinning mechanisms. Several reports utilize different murine models of CRC, which complicates side-by-side comparisons. These contradictory findings on the role of IL-33/ST2 signaling for CRC tumorigenesis makes it difficult to evaluate the suitability of this pathway for CRC therapy.

2.4. Considerations regarding the role of IL-33 in different cancers

Besides the cancer types mentioned above, other studies have revealed conflicting findings on the role of IL-33 for other different types of tumors including head and neck squamous cell cancer (HNSCC) [54–57], skin cancer [58,59], and between different hematological malignancies [60]. In some cancers, IL-33 was so far found to be solely pro-tumorigenic [61–64] or to exert only an anti-tumorigenic effect (AML) [65] (see Table 1).

The conflicting outcomes in these studies may rely on several factors that make comparison between studies difficult – even within the same cancer type. Firstly, within some patient cohorts, the standard treatment of certain cancers include radiotherapy and chemotherapy [32]. This can lead to a rapid tissue increase of IL-33 protein expression [66], thereby leading to confounding findings in the studies where information on the treatments patients received was unclear.

Second, the presence of distinct IL-33- or ST2-expressing cell populations in the microenvironment of these different malignant conditions may influence disease progression [44,47,61,67], as well as it likely the case for species-dependent differences in the expression pattern of IL-33 between human and murine models [25].

Furthermore, for certain tumor entities, *in vivo* models overexpressing ST2/IL-33 may not compare to exogenous addition of (recombinant) IL-33 into tumors. As the bioactivity of IL-33 is tightly regulated and its availability may consequently vary, the dose administered (via exogenous administration) or the amount of over/reduced expression of IL-33 (after genetic manipulation) can differently shape the TME and its immune contexture [43,48], and therefore possibly explain the conflicting findings in the studies mentioned above. In addition, *in vitro* studies focusing on a single type of cell may not faithfully replace the conditions found in a complex organism, and there may be differences between primary cells and (transformed) cell lines.

Experimental approaches designed to minimize confounding factors based on microbiota [68,69], or including blockade at different stages of tumorigenesis or in different cell types may help shed more light on the precise contribution of the IL-33/ST2 pathway to CRC development. Also, identification in the TME of shared versus specific cell types that respond to IL-33, may represent a first step in disentangling the molecular events affecting tumorigenesis in different cancers.

Overall, these various parameters should be considered and possibly

addressed in future investigations addressing the contribution of IL-33/ST2 in cancer, also to allow for easier comparison between studies.

3. IL-33 and immune cell types in the tumor microenvironment

IL-33 may regulate the activation of various immune cell types, which greatly affects the course of cancer or inflammatory disease. Due to its pleiotropic activity, IL-33 may modulate immune populations that are either clearly pro-tumorigenic or strictly anti-tumorigenic. A likely balance between these divergent effects will depend not only on IL-33-mediated stimulation, but also on the general inflammatory milieu, i.e. the other cellular/soluble factors governing activation of these immune cells. However, IL-33 may also elicit in specific immune cell types both pro-tumorigenic and anti-tumorigenic responses (Fig. 1). We review below how IL-33 can shape the immune contexture in the TME and how the immune populations react differently to IL-33.

3.1. Effect of IL-33 on $CD4^+$ T helper cells

Accumulating evidence suggests that the IL-33/ST2 axis plays an

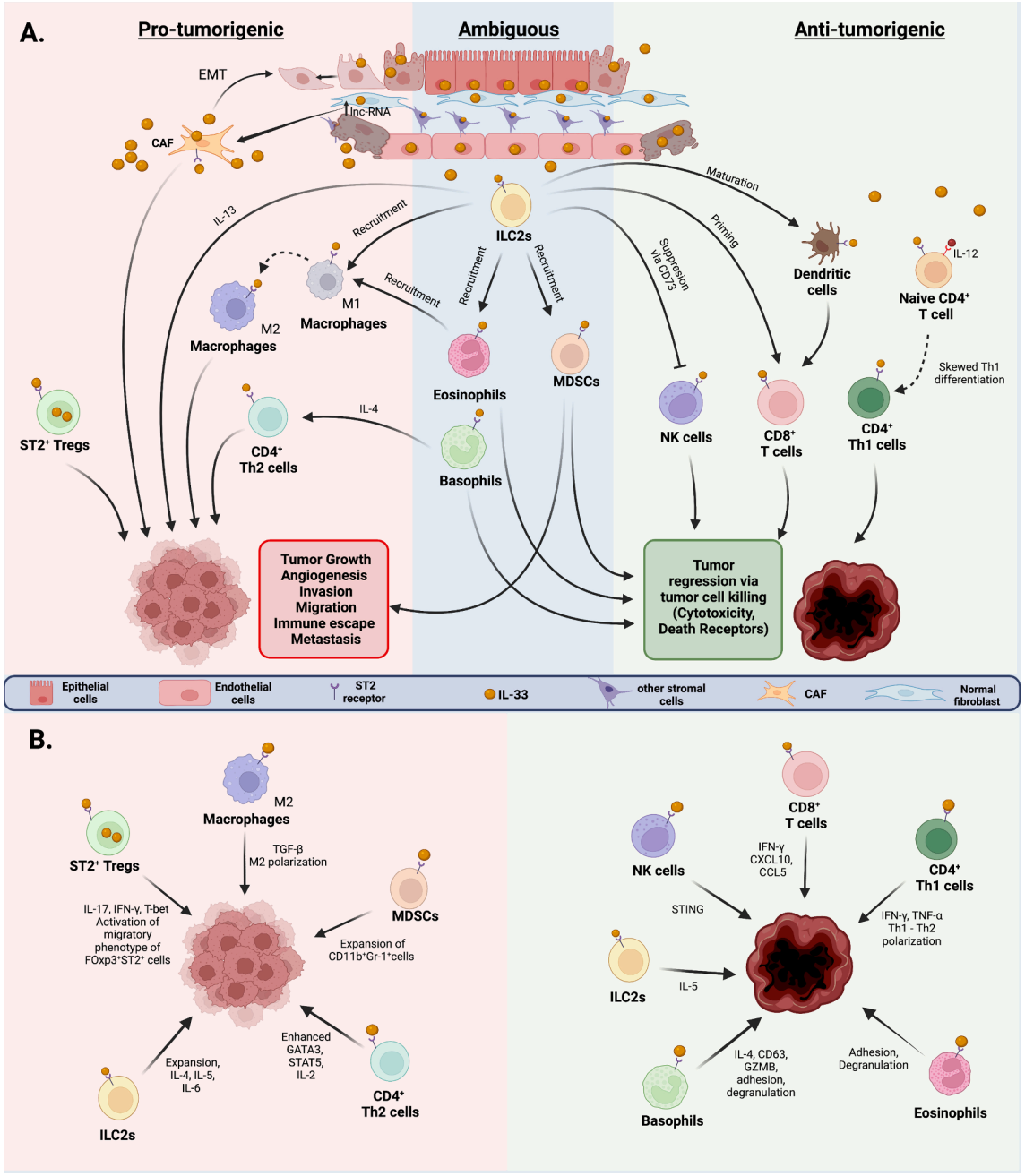

Fig. 1. Anti- and pro-tumoral role of IL-33 in modulating the tumor microenvironment (TME). A) IL-33 is expressed in epithelial cells, endothelial cells, fibroblasts and stromal components such as cancer-associated fibroblasts (CAFs). The release of IL-33 during cell stress or damage, or the increased expression of IL-33 in CAFs maintains/activates suppressor immune cells such as tumor-associated macrophages, Tregs and $CD4^+$ Th2 cells, thus contributing to tumor growth and metastasis. In contrast, IL-33 may also exerts an anti-tumor effect by activating innate (NK) and adaptive ($CD4^+$ Th1, $CD8^+$ T cell) immune responses. However, IL-33R signaling can lead to a dual or ambigious role on other cell types such as eosinophils, basophils, ILC2s and myeloid-derived suppressor cells (MDSCs) either directly or through interaction with other cell types, although this is dependent on disease and cancer type. B) Binding of IL-33 to its receptor ST2 on immune cells induces different biological effects resulting in angiogenesis or tumor regression. This figure was created using Biorender.com.

important immune modulator in the TME, where it acts on immune cell populations associated with type 2 and regulatory immune responses [31,46].

However, recent findings indicate IL-33 may as well induce type-1 immune responses, thereby differently regulating the immune contexture and tumor development in the TME (Fig. 1).

Both conventional naïve and regulatory $CD4^+$ T cells constitutively express ST2 and are thus direct targets for IL-33. IL-33 generally promotes naïve T cells to differentiate into a T helper 2 (Th2) cell phenotype [31]. However, anti-tumor activity is usually attributed to T helper 1 (Th1) cells, which support the recognition and elimination of transformed tumor cells by cytotoxic lymphocytes [31]. Recent studies show that IL-33 may also induce an anti-tumor response by promoting Th1 activity [70]. *In vitro*, IL-33 was able to potentiate and promote the early differentiation of $ST2^+$ naïve T cells into Th1 cells, yet only when used in conjunction with IL-12 [71]. In a Human Papilloma Virus (HPV)-associated mouse tumor model, exogenous IL-33 was found to exert immuno-adjuvant effects by driving IFN-γ and TNFα production by $CD4^+$ T cells, leading to the upregulation of antigen-specific IgG and subsequent tumor regression [72]. Similarly, endogenous IL-33 was reported to enhance expression of IFN-γ^+ in $CD4^+$ T cells in murine models of colon [73] and hepatocellular [74] carcinoma.

An imbalance in the Th2/Th1 ratio has been described to regulate the outcome of several types of cancer [75]. Based on the above-presented studies illustrating the dual capacity of IL-33 in promoting both Th2 and Th1 responses, it is conceivable that IL-33 may have a strategic relevance in shaping the type of $CD4^+$ T helper response within the TME and thereby determining tumor progression. These findings also suggest that IL-33 may act in concert with other cytokines or inflammatory mediators to support their priming function.

3.2. Effect of IL-33 on $CD4^+$ regulatory T cells

Tregs promote a pro-tolerogenic tumor environment supporting tumor growth, and ST2 expression can be found on Tregs in the TME. IL-33/ST2 signaling favors the recruitment of suppressive $CD4^+Foxp3^+GATA3^+$Tregs into the TME and it activates their suppressive function, thus favoring tumorigenesis [46,70]. $ST2^+$ $FOXP3^+$ Tregs are found in the stroma of human CRC patients, where high expression of these two markers hinted toward a shorter overall survival [76] . Using a chemically-induced murine model of CRC based on treatment with azoxymethane (AOM) and dextran sulfate sodium (DSS), our group found that IL-33/ST2 signaling promotes tumorigenesis turning $FoxP3^+$ Treg cells into a more activated, migratory phenotype that favors their accumulation into the TME [44,48]. Another study using a mouse model of lung adenocarcinoma demonstrated that abrogation of Treg-specific *St2* (KPF-$St2^{FL}$ mice) enhanced $CD8^+$ T cell infiltration into cancer tissues, which decreased tumor size and number. This suggests an important role for ST2 in Treg-specific immunosuppression in cancer [77].

In a mouse model of B16.F10 melanoma, abrogation of *Il33* – but surprisingly not *St2* – favored the conversion of tumor-specific, stable Tregs into "fragile" Treg cells, which maintain FOXP3 expression but upregulate T-bet, IFN-γ and mTORC1. This was associated with a loss of Treg suppressive capacity and subsequent tumor regression and indicated a cell-intrinsic role of IL-33 in regulating the stability of Tregs in the TME [78]. Overall, these studies provide evidence that IL-33/ST2 signaling alters Treg activation, phenotype and migration to support their pro-tumorigenic function.

3.3. Effect of IL-33 on cytotoxic lymphocytes

$CD8^+$ T cells and natural killer (NK) cells are cytotoxic effector cells that exert a paramount role against pathogens and cancers [79]. Several studies have established a contribution of IL-33/ST2 signaling to the expansion of effector $CD8^+$ T cells and NK cells in the TME. In mouse models of colon carcinoma and breast cancer, administration of endogenous IL-33 induced an anti-tumor response by promoting the recruitment of $CD8^+$ T and NK cell to the TME [47,80,81].

In the B16 melanoma and Lewis lung carcinoma metastatic models, transgenic expression of IL-33 in tumor-bearing mice stimulated NF-κB signaling to promote the activation of tumor-infiltrating NK and $CD8^+$ T cells [43,82]. During Lewis lung carcinoma, IL-33 was also found to promote dendritic cell differentiation and maturation, which in turn upregulated $CD8^+$ T cell and NK cell tumor immunity [80,83]. IL-33 may also stimulate interferon expression in both $CD4^+$ and $CD8^+$ T cells, which increases anti-tumor $CD8^+$ T and NK responses in different cancer models [73,74,84].

Taken as a whole, IL-33 enhances the effector function of cytotoxic lymphocytes to restrict tumor growth.

3.4. Effect of IL-33 on macrophages

IL-33/ST2 signaling promotes in tumor-associated macrophages (TAMs) the expression of M2-like markers and their immunosuppressive capability. For instance, IL-33 competitive inhibition using a soluble ST2 fusion protein inhibited the polarization of M2-like TAMs therefore restricting the growth of NSCLC xenografts [85]. In agreement with these findings, IL-33 expression in mouse models of CRC and breast cancer induced the recruitment into the TME of M2-like TAMs, hence promoting their pro-tumorigenic function [42,86–88]. In the context of gastric cancer, IL-33 indirectly mobilizes pro-tumorigenic macrophages by activating IL-33-responsive mast cells [89].

Overall, IL-33 effect on macrophages generally leads to a more immunosuppressive phenotype that promotes cancer progression.

3.5. Effect of IL-33 on eosinophils

Eosinophils are granulocytic white blood cells more commonly known to play a role in host defense against parasites and infections. Yet eosinophils have been increasingly reported to invade tumors in human patients and in experimental cancer models, suggesting a relevant role for tumorigenesis [90]. IL-33 was first described to indirectly recruit eosinophils by inducing IL-5 production [91]. However, subsequent reports established that IL-33 can also directly act on eosinophils to regulate their development, survival, activation and adhesion [92,93]. In different melanoma mouse models, tumor expression [58] or injection [94] of IL-33 resulted in delayed tumor growth, where this anti-tumorigenic effect was dependent on eosinophils [58]. Furthermore, in CRC models based on CT26 cell engraftment or chemically-induced intestinal tumorigenesis, adoptive transfer of eosinophils activated *ex-vivo* with IL-33 inhibited tumor growth [95].

Mechanistically, IL-33-activated eosinophils exerted their anti-tumor activity both through a direct cytotoxic effect against tumor cells or by playing an accessory role, through the release of pro-inflammatory factors activating $CD8^+$ T cells [58,95–97].

However, IL-33 also activates eosinophils to promote downstream recruitment of macrophages, thereby supporting metaplasia within the gastrointestinal tract [98].

3.6. Effect of IL-33 on basophils

Basophils constitute a rare cell population that have been found in the TME of certain cancers [99]. Early reports on basophils in cancer suggest a dual role of basophils, where these cells may either support or inhibit tumorigenesis upon IL-33 activation [99].

IL-33 can activate ST2-expressing murine and human basophils, which stimulates *in vitro* their cytokine production and histamine secretion and *in vivo* their proliferation [93,100]. IL-33 alone does not directly induce human basophil degranulation; however, it can potentiate production of IL-4 upon IL-3 stimulation or IgE cross-linking [97,100,101]. Moreover, IL-33-mediated activation of basophils

promoted expression of the degranulation marker CD63 and of granzyme B, which led to tumor cell killing *in vitro* [99]. On the other hand, basophils were found to be a relevant source of IL-4 in pancreatic ductal adenocarcinomas (PDAC), promoting a pro-tumorigenic Th2 response in the TME. Hence, IL-33 mediated release of IL-4 or degranulation in basophils could be relevant in modulating cancer progression [102]. However, the role of basophils, as well as its response to IL-33, in the TME remains unclear.

3.7. Effect of IL-33 on myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) comprise of a heterogeneous group of immature myeloid cells that are absent in healthy conditions, but expand during cancer, infection and inflammatory disease where they suppress the immune response [103]. Various studies have indicated a positive effect of IL-33/ST2 signaling on MDSC numbers during tumor progression. For instance, administration of exogenous IL-33 in a 4T1 model of murine breast cancer promoted systemic and intratumoral expression of $CD11b^{+}Gr\text{-}1^{+}$ MDSCs [67]. Consequently, *Il33*-deficient mice showed diminished accumulation, proliferation and immunosuppressive activity of MDSCs [42,67]. Along these lines, IL-33 also enhanced MDSC recruitment in CRC, leading to increased tumor angiogenesis and metastasis via remodeling of the TME [104].

However, conflicting results have been also described, indicating that IL-33 inhibited the differentiation and immunosuppressive activity of MDSCs *in vitro* [105]. Moreover, administration of IL-33 into a heterotopic mouse model of melanoma counteracted and impeded the accumulations of MDSCs in the spleen and TME [58,105]. These contradictory results suggest that IL-33 may either promote or inhibit the expansion/recruitment of immunosuppressive MDSCs depending on the tumor type or possibly the source of IL-33.

3.8. Effect of IL-33 on type 2 innate lymphoid cells

Type 2 innate lymphoid cells (ILC2s) belong to the family of innate lymphoid cells that lack antigen specific B and T cell receptors. ILC2s react to stimuli by traditionally producing type 2-associated cytokines, hence their name. $ST2^{+}$ ILC2s can be directly triggered by IL-33, which leads to their activation and expansion [106]. ILC2 may have pleiotropic functions including both inflammatory and tissue repair activities [107]. Recently, ILC2s have been increasingly investigated for their role in regulating tumorigenesis [108,109], where current findings indicate both pro- and anti-tumorigenic effects concordant with their pleiotropic functions.

IL-33-activated ILC2s have modulatory effects on MDSCs [110,111], cytotoxic T lymphocytes / NK cells [112–114], and eosinophils [115,116], leading to either pro- or anti-tumorigenic outcomes. This is mainly mediated through ILC2-dependent production of IL-5 and IL-13, as well as purinergic regulation of NK cells via CD73 on ILC2s [114]. For instance, a recent study reported an IL-13 mediated pro-tumoral response of ILC2 in a murine model of CRC, which involved an IL-33/ST2/IL-13 axis in ILC2s that was under the control of peroxisome proliferator-activated receptor gamma (PPARγ) [117]. On the other hand, IL-33 can expand and activate tumor-infiltrating ILC2s, thus promoting their anti-tumorigenic activity via ILC2-mediated direct priming of $CD8^{+}$ T cells or indirectly via the recruitment of $CD103^{+}$ dendritic cells in pancreatic carcinoma [112]. In addition, in a mouse model of melanoma, IL-33-mediated activation of ILC2s led to reduced tumor growth via IL-5 mediated eosinophil recruitment, while tumor-derived lactate production impaired ILC2 function [116,118].

Overall, IL-33-mediated stimulation of ILC2s plays a role across all stages of inflammation and affects a variety of immune cells relevant in regulating tumor growth. In the context of cancer, a similar range of modulating effects can be observed, whereby ILC2s are likely involved in the crosstalk between a variety of cell types, which may explain the different disease outcomes depending on the tumor type and context.

4. IL-33 expression and activity on stromal cells in the tumor microenvironment

4.1. IL-33 expressing cells in the tumor microenvironment

The contribution of IL-33 to different pathologic conditions is determined by its cellular source in the TME. Stromal cells are the main non-immune cells responsible for stimulating proliferation of the malignant clone, where they represent a major source of IL-33 both at steady-state and during disease [5,119]. Thus, it is relevant to characterize and distinguish the effect of IL-33 originating from both stromal, non-tumor cells, versus from tumor cells, e.g. from the transformed epithelium.

Transformed (epithelial) tumor cells constitute an important source of IL-33 in the TME. Tumor-derived IL-33 promotes activity of resident $CD8^{+}$ T cells, both directly and indirectly via dendritic cells, which provide a supportive role during immune checkpoint blockade therapy in different murine models of solid cancers [120]. Considering that IL-33 usually functions in close proximity to where it is released, tumor-derived IL-33 likely affects anti-tumor cytotoxic immune cells in the vicinity.

Fibroblasts are a major constituent of the TME, whose dysregulated function during cancer may promote a tolerogenic environment favoring tumor growth through extracellular matrix remodeling and the release of pro-angiogenic factors [121]. A microarray analysis of organotypic cultures of HNSCC revealed cancer-associated fibroblasts (CAFs) as high expressers of IL-33 [54]. Correlative studies using The Cancer Genome Atlas (TCGA) in melanoma and HNSCC suggest that stromal-derived IL-33 was associated with tumor metastasis [56,59]. Upregulated IL-33 in CAFs increased lung metastasis in a mouse model of breast cancer, via modulation of the immune system and subsequent establishment of inflammatory niche in the lung that aids metastasis [122]. This demonstrates that CAF-derived IL-33 not only alters the microenvironment surrounding the tumor, but that it may also affect other organs via its action on the immune system. Moreover, epithelium-derived IL-33 was a driver of CRC tumorigenesis in $APC^{Min/+}$ mice [123].

On the other hand, mRNA sequencing data retrieved from TCGA indicated that IL-33 expressed in endothelial and epithelial cells, instead of fibroblasts, in HNSCC and melanoma could favor anti-tumor immune responses [56,59].Undeniably, further studies targeting IL-33 in specific cell types would help determine the relevance and the relative contribution to disease of IL-33 from different cellular sources.

4.2. Effects of IL-33 on stromal cells in the tumor microenvironment

IL-33 in the TME not only alter immune cell function; it may as well stimulate non-hematopoietic cells. For instance, in the $APC^{Min/+}$ murine model of CRC, IL-33 stimulated $ST2^{+}$ fibroblasts to produce pro-tumorigenic factors supporting TME remodeling, including extracellular matrix components and growth factors [47]. In SHIP1-deficient mice, myeloproliferative disease was triggered by pro-tumorigenic cytokines and growth factors released by bone marrow stromal cells upon stimulation with IL-33 [61]. Therefore, stromal cells in the TME not only provide a source of but may also respond to IL-33, possibly in an autocrine manner, to promote tumor development.

A lesser-studied function for IL-33 includes a possible role in regulating epithelial-to-mesenchymal transition (EMT), a process that is necessary during development and tissue repair, but also for the invasiveness and metastatic activity of solid tumors. Through ST2 signaling and CD146, IL-33 was able to trigger EMT during airway remodeling in the context of chronic allergic inflammation [124]. IL-33 induced EMT via TGFβ/SMAD signaling in lung epithelial A549 cells and in small airway epithelial cells [125]. Similarly, IL-33 may drive an endothelial-to-mesenchymal transition (endoMT) via ST2 signaling in the context of development or regeneration after injury [126]. Further evidence of this unconventional IL-33 function in promoting cell transition to a

mesenchymal has been also provide in the context of cancer. For instance, IL-33/ST2 signaling activated the JNK pathway to increase the cell migration, invasion, EMT and stemness of tumor glioma cells [87]. In the adenocarcinoma cell line HT29, exogeneous IL-33 was able to regulate gene transcription markers associated with EMT, where it subsequently led to increased migration and a migratory mesenchymal phenotype [127].

IL-33 expressing CAFs enhanced the migratory and invasive capacity of co-cultured $ST2^{+}$ gastric cancer cells by inducing EMT through activation of an ERK1/2-SP1-ZEB2 pathway [64]. These different studies indicating a contribution of IL-33 to tumor progression and metastasis, suggest this cytokine to be a central determinant of clinical outcome in cancer patients.

5. Dynamic regulation of IL-33 – How and why it matters

In light of the numerous discrepant studies on the role of IL-33 in cancer, several aspects of IL-33 biology and function need to be re-explored in further detail to explain these inconsistencies. Thus, next we aim to discuss possible mechanisms underpinning these discrepancies, including molecular checkpoints controlling IL-33 availability. For instance, post-transcriptional regulations or modifications can substantially contribute to the overall effect and potency of several proteins in general, and of IL-33 in particular. Therefore, it is important to consider how mechanisms regulating IL-33 level and activity to eventually understand the net effect of IL-33 on its target cells.

While IL-33 expression tends to be constitutive in several cell types [128,129], intracellular IL-33 expression has also been seen to be increased [129–135] or decreased [5,136,137] depending on the cell type and the disease context. Current studies suggest that there are multiple post-transcriptional secretory or regulatory pathways associated with IL-33 stability, secretion and maturation, where transcription level of the *IL33* gene might not always reflect IL-33 protein level or activity. This might have a slight but significant contribution in determining cancer outcomes and explain why there are differences concerning the role of IL-33 both within and between cancers.

5.1. Post-transcriptional regulation of IL-33 protein levels

The nuclear sequestration of IL-33 has mainly been explained through its tight association with chromatin via its chromatin-binding domain [138]. Regulation and retention of nuclear IL-33 levels likely play a crucial effect on the potency of this cytokine upon release.

IL-33 retention was associated with importins – chaperone proteins involved in nuclear transport, protein stability and protein secretion [139]. More specifically, importin 5 was shown to be a binding partner of nuclear flIL-33, but not mIL-33, where it is able to protect IL-33 from proteasome-mediated degradation in the nucleus [139]. Possible negative regulators of this IL-33 degradation are USP17 and USP21, ubiquitin-specific proteases that can de-ubiquitinate nuclear IL-33 and thus maintain stability of the nuclear protein [140,141]. USP17-mediated de-ubiquitination of nuclear IL-33 was responsible for regulating the DNA-binding function of IL-33 [141]. Interestingly, several USPs, including USP17 and USP21, are dysregulated in different cancers and critical to cancer progression and metastasis [142–144]. This may suggest a role for USPs affecting cellular transformation via IL-33. Since ubiquitination of IL-1β, a related IL-1 family cytokine, leads to its proteasomal degradation as well as limits its cleavage by caspase 1 [145], ubiquitinated IL-33 might undergo similar regulatory mechanisms.

One can speculate that, at steady-state, turnover of IL-33 protein might occur at a rapid rate via ubiquitination-mediated proteasomal degradation, where ubiquitination of IL-33 also serves to suppress its nuclear function via inhibition of binding to its target DNA site. Dysregulated protein turnover is a hallmark of cancer [146], whereby this dysregulation might contribute to abnormal IL-33 protein levels, which may have an impact on the potency/activity of IL-33. Yet, aside from regulating IL-33 protein quantity, protease-mediated cleavage can also modulate IL-33 function and bioactivity.

5.2. IL-33 release and secretion

Due to lack of a secretion sequence, IL-33 is commonly thought to be released into the extracellular environment only during cell necrosis/damage [2], hence its original classification as an alarmin. However, this dogma has recently been challenged, as living cells were found to secrete IL-33, whereby the exact mechanism for this release remains unclear. While flIL-33 is bioactive only at high concentrations, lower concentrations of flIL-33 in the milieu may still function as a sensor and cell activator after being extracellularly cleaved by environmental allergen proteases [147]. This may indicate a conserved functional role of flIL-33 in regulating immune responses during steady-state conditions despite its low physiological concentration. As such, current studies show that flIL-33 can be released from the nucleus of different cell types upon mechanical stress or other unknown stimuli, even in absence of cellular necrosis. Upon triggers including mechanical stress [148] or nigericin (via calcium signaling) [149], nuclear IL-33 seemingly transits to the cytoplasm via the nuclear pore, while residing in membrane bound-vesicles to facilitate extracellular secretion from either live or dying cells. However, the mechanism of extracellular release of these IL-33-containing vesicles is still ambiguous.

It was shown that flIL-33 release from live macrophages was not dependent on caspase-1, caspase-8 or calpain cleavage, suggesting a different secretion process compared to other cell types [150]. Passive release of flIL-33 in macrophages might depend on certain pore-forming proteins, such as perforin 2, which is constitutively expressed in these cells. This is supported by a study showing IL-33 from live dendritic cells being released through a pore formed by perforin 2, with perforin 2 being upregulated in an IL-33/ST2 dependent manner to make a feed-forward loop [151]. Gasdermin pore formation may potentially facilitate this extracellular release during inflammatory cell death (pyroptosis) [152,153], in a similar way as for IL-1β, with recent findings in other contexts supporting this unconventional secretion [153–155].

A recent study suggests that, in chronic obstructive pulmonary disorder (COPD) patients, IL-33 accumulated in the cytoplasm of airway epithelial cells can be co-secreted with exosomes [156]. Alternatively spliced IL-33 isoform $IL\text{-}33^{\Delta exon3,4}$, mentioned above to be relevant in asthmatics [157,158], was able to be more efficiently secreted with exosomes compared to flIL-33, likely due to its inherent cytoplasmic localization [156]. Strikingly, IL-33 secreted with exosomes is surface-bound, yet the mechanism behind this binding is still unclear. It is unknown if such type of release increases the potency or stability of IL-33, but it further highlights the complexity of IL-33 maturation and its availability in the extracellular space. This form of exosome-dependent IL-33 secretion from living cells implies that flIL-33 may be processed post-transcriptionally to be released from chromatin for extracellular transport. Here again, the molecular events controlling flIL-33 or mIL-33 secretion from living cells are still elusive.

Overall, these data support the notion that while IL-33 predominantly functions as an alarmin that is liberated upon necrosis/damage, it may also have a role as a traditional extracellular cytokine secreted by living cells during steady-state, or upon cellular activation.

5.3. Post-transcriptional regulation of IL-33 protein activity

The cytokine activity of IL-33 is mainly regulated by its sequestration in the nucleus, where altered subcellular localization can lead to non-resolving lethal inflammation [11]. However, chromatin binding to IL-33 might not only serve to sequester IL-33 in the nucleus; it may as well enable slow and durable extracellular release of flIL-33. Upon release, IL-33 bioactivity in the extracellular milieu is increased via bound histones [159]. This finding suggests that cells can maximize IL-33 function by extending its duration of release and upregulating IL-33

potency in the milieu. This might delineate a difference of potency between actively secreted IL-33 and cell necrosis/cell damage-mediated release of IL-33.

The current paradigm of IL-33 maturation refers to flIL-33 being extracellularly cleaved into mature, highly bioactive IL-33 isoforms via serine proteases (produced by neutrophils and mast cells) upon acute necrosis [9,10]. Yet the very same proteases that regulate this maturation process can also suppress IL-33 responses through degradation at the IL-33R/ST2 binding domain [160]. In addition, oxidation of extracellular IL-33 by free cysteines can also diminish ability of IL-33 to engage ST2 [12]. Moreover, during apoptosis, IL-33 was thought to be inactivated via caspases involved in mediating the apoptotic pathway to prevent inappropriate immune responses [161,162]. These regulatory mechanisms are hypothesized to limit the activity/aggregation of IL-33 in its immediate surrounding, which may lead to exacerbated inflammation. However, new findings suggest that the post-transcriptional regulation of IL-33 activity is more complex than initially anticipated, with multiple mechanisms for producing different IL-33 isoforms with different bioactivities.

5.3.1. Messenger RNA alternative splicing

One way IL-33 isoforms may be generated is via alternative splicing of pre-mRNA. Multiple splice variants of IL-33 have been reported and described to vary in their expression depending on the cell type [163]. One of these possible splicing events leads to the formation of an IL-33 isoform lacking exons 3 and 4, named IL-33$^{\Delta exon3,4}$. The IL-33$^{\Delta exon3,4}$ isoform was previously described to have constitutive activity *in vitro* [157], whereby deletion of exons 3 and 4 confers cytoplasmic localization and facilitates extracellular secretion of IL-33 [158]. Alternatively spliced IL-33$^{\Delta exon3,4}$ is present in the lung at steady-state, while also highly associated with type 2 inflammation [156,158].

Transposable elements (TE)-driven transcription of the *IL33* gene can also generate a novel isoform of a chimeric IL-33 transcript (LTR-*IL33*), which is expressed in a subset of colon cancer samples. LTR-*IL33*, which lacks the initial N-terminus and several conserved residues of flIL-33, plays a specific role in optimizing the 3D growth of the CRC cell lines HT115 and LS513 [164].

These examples illustrate that mRNA alternative splicing of IL-33 is biologically relevant and should therefore be further examined in the context of cancer and inflammatory diseases.

5.3.2. IL-33, caspases and cell death

Intracellular proteolytic cleavage is another major regulator of IL-33 maturation since this cytokine contains possible cleavage sites. Caspases, with their role as major intracellular proteases, were initially prime suspects of being involved in activating IL-33, also due to their well-described role for the cleavage/maturation of other IL-1 family cytokines (e.g. IL-1β, IL-18) [165]. Yet, the current literature indicates that caspases, as mediators of cell death, rather inactivate IL-33 to prevent inappropriate inflammation during apoptosis. Indeed, proteolytic cleavage of IL-33 by caspases 3/7, downstream of the apoptotic mechanism led by caspase 8, seemingly inhibits IL-33 during cellular apoptosis [161,162].

However, recent findings have challenged this simplistic view of the IL-33-inactivating function of caspases. During environmental allergen challenge, caspase 3/7 is able to process nuclear flIL-33 into a bioactive stable isoform following formation of a ripoptosome, without the traditional use of neutrophilic proteases in the milieu [7]. Formation of the ripoptosome usually precedes cell death and can lead to a form of necroptosis [166], a more inflammatory form of apoptosis.

Other forms of cell death can be triggered following failure of apoptosis [167]. Catalytically-inactive caspase 8 regulates the formation of the inflammasome and leads to canonical activation of caspase 1 during pyroptosis, another inflammatory variant of cell death [167]. However, caspase 1 can initiate apoptosis in gasdermin D-low/null cell types, suggesting roles in regulating non-inflammatory cell death (apoptosis) and pyroptosis [168]. While IL-33 maturation was initially shown to be caspase-1 independent [161,169], several further studies suggest caspase-1 cleavage of IL-33 leads to flIL-33 inactivation *in vitro* [170] and *in vivo* during house dust mite (HDM) treatment [132], in agreement with the IL-33-dampening effect of intracellular caspases. On the other hand, caspase-1 may also upregulate IL-33 physiological activity *in vitro* [2] and *in vivo* [171,172] in a murine model of alum adjuvant treatment or asthma exacerbation. Furthermore, during dry eye disease, IL-33 processing was regulated by caspase 1 cleavage during gasdermin D-driven pyroptosis, resulting in IL-33 activation and release [153].

Further proteases also participate in the regulation of IL-33 bioactivity. For instance, calpain, a calcium-dependent cysteine protease involved in many different cell death mechanisms such as apoptosis and necroptosis, can also cleave and activate IL-33 [8,173]. While it does not play a dominant role in IL-33 maturation during *A. alternata* infection, dysregulated calcium signaling leading to aberrant signaling and inappropriate upregulation of calpain are highly implicated in tumorigenesis [174], where calpain might mediate downstream outcomes via IL-33.

Considering the role of caspases and calpain in mediating different forms of cell deaths, it is possible that the activation/inactivation of IL-33 based on caspase-mediated cleavage is more reflected on the type of cell death, rather than on one specific caspase - i.e. with enhancement during pyroptosis, or suppression of IL-33 activity during apoptosis. Yet the underpinning mechanisms as to how a same caspase can lead to differential bioactivity or isoforms of IL-33 is still unclear. There may be certain factors facilitating the cleavage of different sites of IL-33 during cell death types, consequently leading to different isoforms/bioactivity of IL-33.

This protease-induced regulation of IL-33 is further complicated by the observation that multiple mature forms of IL-33 can be found during epithelial damage / *A.alternata* infection, which is consistent with some, but not all, proposed cleavage sites [8]. Furthermore, allergen protease-mediated cleavage of mouse flIL-33 and human flIL-33 resulted in a rapidly degrading or more stable form of mIL-33 respectively, which are likely depending on differences in the amino acid sequence [147]. This suggests there are further mechanisms or aspects of IL-33 proteolysis that have not been mechanistically explored yet. Interestingly, while short incubation of neutrophil derived proteinase 3 (PR3) lead to IL-33 activation, longer incubation time with this enzyme resulted in multi-site cleavage and subsequent inactivation of IL-33 [160]. Such a time-dependent effect on IL-33 maturation might also be relevant in caspase-mediated cleavage of intracellular IL-33 and explain inconsistencies in the literature concerning activation/inactivation of IL-33 by the same caspase.

As different IL-33 isoforms show variable efficacies in binding to several polymorphic forms of ST2 [175], this emphasizes the importance of understanding the specific function of the various isoforms expressed in distinct cell types and disease contexts.

Another aspect that might explain the multiple discrepancies on IL-33 action in certain cancers might be due to the different capabilities of the malignant cells to undergo a specific type of cell death. As the mechanisms involving cell death are dysregulated in cancer, it is likely that downstream IL-33 maturation, as a consequence of altered caspase activity, would also be affected. One can speculate that, considering the heterogeneity of tumor cells and progressive loss of cell identity, the IL-33 isoforms released from different tumor cells would vary. These considerations highlight the importance of scrutinizing the source of IL-33, as the potency or bioactivity of IL-33 will differ based on its maturation in different cell types as well as their propensity for undergoing inflammatory versus tolerogenic cell death pathways.

5.4. Determination of IL-33 activity by the cellular context

Distinct cell types exhibit different IL-33 responses depending on the disease context. In a model of HDM-induced allergic rhinitis, it was

observed that tissue-derived IL-33, but not immune cell-derived IL-33, mediates the induction of airway inflammation [176]. On the other hand, while *Il33*-deficient mice exhibited a failure to clear *N. brasiliensis* infections [177], epithelial-derived IL-33 had opposite roles compared to dendritic cell-derived IL-33 in the immune response during worm clearance [151]. In these two studies, it appears that IL-33 plays a stronger role in activating innate immunity due to its close interaction with the barrier cells in allergic rhinitis, whereas during parasitic infection, worm clearance was more dependent on IL-33 in promoting adaptive immunity. Therefore, there is a context-specific, varying contribution of IL-33 to the different cell populations involved in a particular disease. This may be explained by the short half-life of IL-33 – which restricts its effect on neighboring cells – or the availability of distinct IL-33 isoforms in different cell types. Along the same lines, a correlative analysis based on TCGA mRNA sequencing data indicated that the prognostic value of IL-33 for HNSCC was heterogeneous among tumors from different sites. This led the authors to hypothesize that the cellular source of IL-33 in the tumor microenvironment may determine the distinct roles of this cytokine in HNSCC and melanoma [56,59].

These different reports indicate that while $CD45^{+}$ hematopoietic cells are not a major source of IL-33 even after stimulation, low albeit functional levels of IL-33 can still modulate the immune response and disease outcome [151]. They also illustrate that the relative contribution of IL-33 to immunomodulation can differ depending on the cell populations it originates from and the identity of its cellular targets, and they highlight that the pro- or anti- inflammatory properties of IL-33 are highly context-dependent.

Taken together, IL-33 expression and activity is dynamic, with multiple levels of post-transcriptional mechanisms dictating its accumulation in the nucleus, release in the extracellular space and maturation (Fig. 2). Based on these multilevel regulatory mechanisms of IL-33 expression, release and biological activity, studies with correlative analysis between IL-33 and disease outcomes in cancer should be carefully interpreted both for past and future research. This applies in particular to inferences on the contribution of IL-33 concentration in serum or in bulk mRNA analyses (from heterogenous cell populations in tissues), where IL-33 levels might not be reflective of the pathogenesis of a particular disease. This emphasizes the need for basic researchers to use cell-specific upregulation or knockout of IL-33 in pre-clinical models of cancer.

Moreover, approached relying on supra-physiological concentrations of IL-33, i.e following systemic intravenous administration or during local endogenous overexpression via genetic manipulation, will very likely induce experimental biases given the important of the local versus systemic cellular context for IL-33 activity.

Differences in IL-33 expression patterns between distinct species such as mice and humans [129], as is it for instance the case for endothelial cells, should be also considered for cancers, since this may lead to

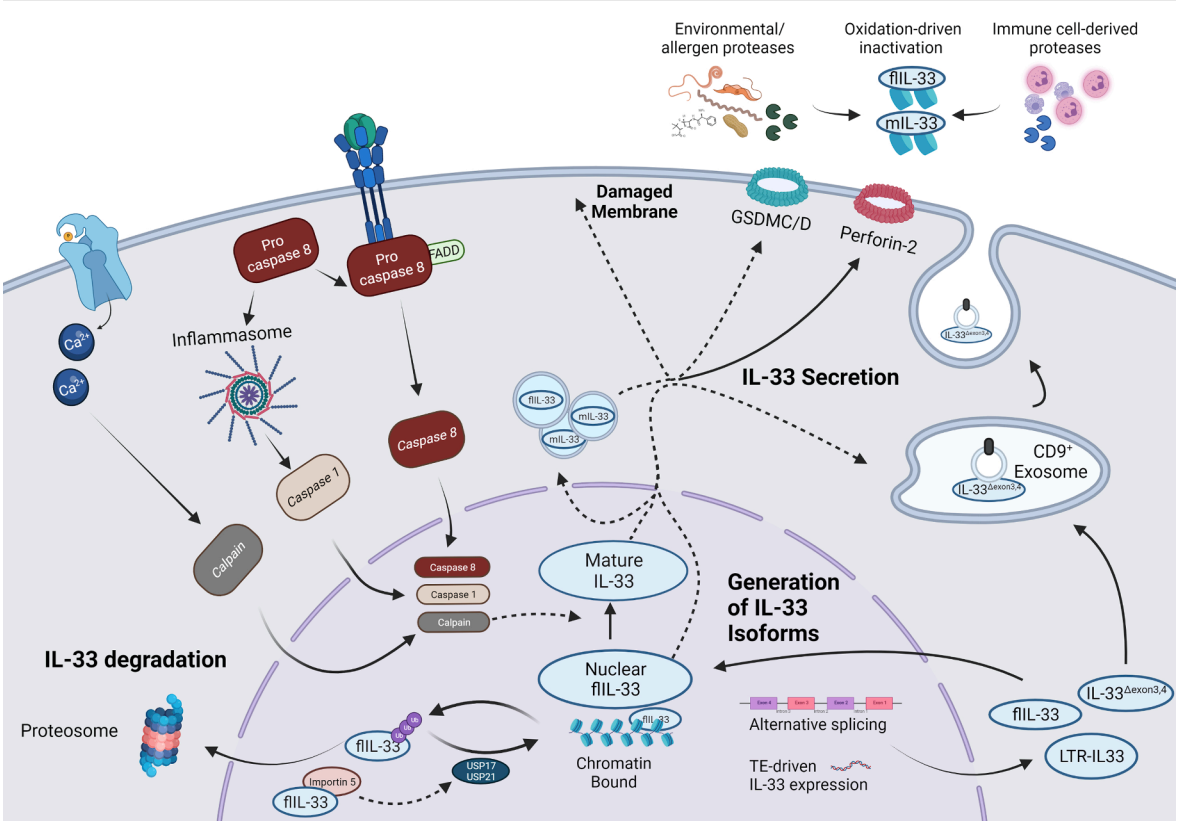

Fig. 2. Post-transcriptional mechanisms regulating IL-33 protein level and activity. Nuclear full length IL-33 (flIL-33) is generally bound to chromatin, which thus sequesters it in the nucleus. Via mRNA alternative splicing ($IL\text{-}33^{\Delta exon3,4}$) or transposon mediated transcription (LTR-IL33), several different protein isoforms of IL-33 along with conventional flIL-33 can be generated, several of which have been associated with inflammatory disease or cancer. Degradation of nuclear IL-33 can occur via ubiquitination and is regulated by ubiquitin-specific proteases (USPs) and importin 5. Upon integration of stimuli/signals, different caspases can become activated depending on the type of cell death (apoptosis, necroptosis, pyroptosis) that is induced, whereby these intracellular proteases may differently process flIL-33 into active or inactive mature IL-33 (mIL-33) isoforms. FlIL-33 and mIL-33 can be released from the cell upon necrosis/cell damage via damaged plasma membranes, but also by live cells. While the mechanism for IL-33 secretion from living cells is still unclear, several secretion pathways have been identified (perforin2 and exosomes, with IL-33 as surface bound protein). In addition, gasdermin (GSDM)-mediated pores might offer an unconventional pathway for release. In the extracellular milieu, histones bound to IL-33 can potentiate binding to the IL33 receptor ST2. Oxidation of cysteine residues on IL-33 can rapidly degrade extracellular IL-33. Cleavage of flIL-33 into active mIL-33 isoforms can occur via environmental or immune proteases in the extracellular space. GSDM: Gasdermin; LTR: Long terminal repeats; TE: Transposable elements. This figure was created using Biorender.com.

differential activation of ST2-expressing cells.

Overall, better delineating the sources, pathways and bioactivity of IL-33 in different cell populations will enable us to understand why discrepancies in the literature regarding the role of IL-33 in tumorigenesis and cancer progression occur, and possibly allow us to evaluate new targets for therapies to regulate IL-33 activity.

6. Other confounding factors of IL-33 activity

6.1. Nuclear functions of IL-33

Current studies on IL-33 mainly focus on the role of IL-33 as an extracellular alarmin, where most studies in the context of inflammatory disease or cancer show concordant results between *Il33*-deficient mice and animals with genetic disruption of *St2*. However, due to its nuclear localization, a putative role of flIL-33 as a regulator of the chromatin landscape or gene transcription has been speculated. Indeed, nuclear flIL-33 has been reported to affect the outcome in certain experimental settings via an unclear ST2-independent mechanism [72,137,141,178–180,181–183].

Several studies have shown that nuclear flIL-33 is involved in the tuning of several intracellular cell signaling pathways, such as NF-κB signaling [78,184–187], JAK-STAT signaling [188,189], and SMAD signaling [190]. Nuclear IL-33 shows different modes of action [78,137,185,186,188], which are illustrated in Fig. 3. This additional level of IL-33-dependent regulation in the nucleus adds some complexity to the role of IL-33 as a soluble cytokine or alarmin in the context of cancer, where intrinsic nuclear IL-33 might possibly also play a minor, yet relevant, role in impacting cancer outcomes by tweaking key cancer-associated signaling pathways [191–193]. An important point to note is that this area of research is still a developing field, where there have been comprehensive studies that support [183,188,190] or disproves [159,194] a moonlighting role of nuclear IL-33. As of today however, the bulk of the literature indicates that the predominant function of IL-33 is the one as an extracellular cytokine, and that the contribution to disease by the nuclear activity of IL-33 is likely minor or at least less frequent. However, nuclear IL-33 may possibly have a bigger role to play in "cold" rather than "hot" tumors, where its action on the stromal/cancer cells would have a larger effect on tumor growth rather than its cytokine activity – and with the latter function being possibly more important in TME characterized by the presence of multiple immune effectors. Nevertheless, this additional function of IL-33 in the nucleus should be taken into consideration while interpreting any discrepant findings both within and between cancers.

6.2. Soluble ST2 – More than a decoy receptor?

The IL-33-binding moiety of the IL-33 receptor, ST2, has been extensively investigated in regard to its cellular expression pattern and to the signaling pathway it conveys [6,195,196]. The ST2-encoding gene *IL1RL1* has been reported to give rise to four different splice variants: ST2L, soluble ST2 (sST2), ST2V, and ST2LV [197,198]. ST2V is highly expressed in gastrointestinal organs and is plasma membrane-bound [197], whereas ST2LV has not been further examined. Interestingly, sST2 might also function as an extracellular ligand by binding to an unknown receptor, suggesting a secondary function as a inflammatory mediator or growth factor instead of a decoy receptor [199,200].

ST2V and sST2 were shown to be decreased in tumor lesions

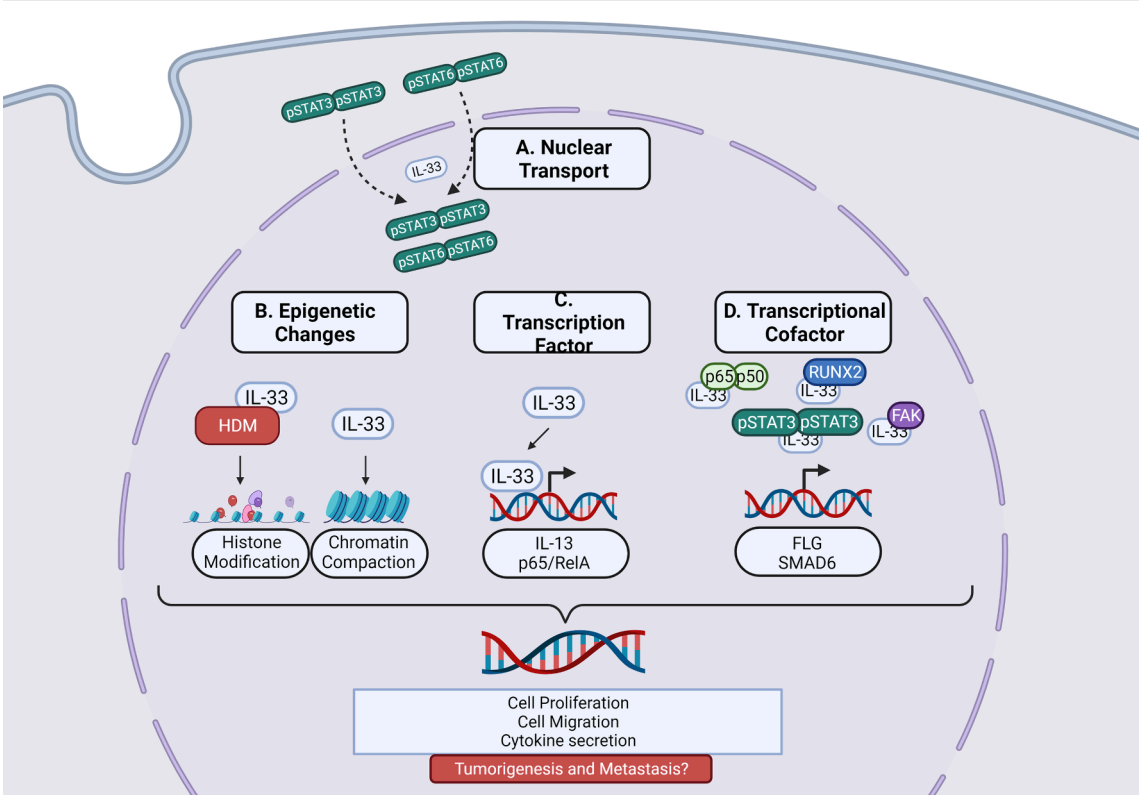

Fig. 3. Role of nuclear IL-33 in regulating cellular activity. A) IL-33 is involved in the nuclear translocation of pSTAT3 and pSTAT6 homodimers. B) IL-33 can cause epigenetic changes through modulating histone methylation and promoting nucleosome-nucleosome interaction, influencing chromatin accessibility to transcription factors. C) IL-33 can directly bind the promoter regions of *IL13* and the NF-κB subunit p65/RelA, thereby driving transcription of these genes. D) IL-33 can serve as a (transcriptional) cofactor of different transcription factors (p65, RUNX2, pSTAT3, FAK) and either inhibit or enhance their binding activity to their target site. Some of the genes identified to be regulated through this function is *FLG*, regulated by an IL-33/pSTAT3 complex and *SMAD6*, that is regulated by an IL-33/RUNX2 complex. These different nuclear functions of IL-33 highlight its contribution to the regulation of cell proliferation, migration and activation, all function, which may have relevant roles in tumorigenesis/metastasis or for immunoregulation in cancer. This figure was created using Biorender.com.

compared to adjacent healthy tissues in a cohort of gastric cancer patients [201]. A possible subsidiary role of sST2 and the obscure function of ST2V might imply an uncoupling of ST2 from IL-33 activity in certain contexts.

7. Concluding remarks

Since its discovery in 2003 (1) and its initial functional description as a cytokine that induces type 2 immune responses through activation of ST2 (2), great strides were made in our understanding of IL-33 biology. Yet here are still many aspects of it that remain unclear and are yet to be addressed. Accumulating data indicate the importance of considering IL-33 as a short-lived, highly potent paracrine molecule, whereby the cellular context of IL-33 expression and release may lead to opposite biological outcomes. This pleiotropic nature of IL-33 is further complicated by the fact that this cytokine or its IL-33/ST2 signaling axis display both extra- and possibly intracellular activities, with layers of tight regulation to suppress or promote IL-33 function.

Several aspects need to be considered to elucidate the reasons for the multiple conflicting reports on IL-33 function in certain disease types and for the development of possible therapeutic strategies for modulation of IL-33/ST2 signaling. 1) The pleiotropic nature of IL-33 with effects of immune cells, stromal cells and cancer cells makes it particularly arduous to delineate its spatiotemporal contribution to tumorigenesis. 2) While still controversial, a role of IL-33 in the nucleus is emerging, where its relevance in determining outcomes in cancer is still unclear. 3) The evidence for post-transcriptional mechanisms regulating IL-33 function, which should be kept in mind to avoid erroneous conclusions, especially when interpreting the role of IL-33 in complex disease pathologies. Of particular interest is for instance the generation of IL-33 isoforms via intracellular proteases associated with distinct types of cell death. Identification and discrimination of these IL-33 variants in different disease settings underline their relevant and differential role for immunomodulation. Delving further into characterizing these IL-33 variants in different human and experimental tumors is certainly warranted.

It is conceivable that several of these regulatory mechanisms are relevant across cancers, and further investigations are needed to elucidate whether they are interconnected to regulate IL-33/ST2 biology. Additional studies will help discern the rationale for the inconsistencies on the precise role of IL-33 in specific disorders. Undeniably, the above-mentioned parameters should be considered and integrated into treatment designs, to evaluate both the “when” and “how”, provided IL-33/ST2 is to be targeted in cancer – or in inflammatory disease – possibly in synergistic combination with existing treatments.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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