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IL-33/IL-31 Axis: A New Pathological Mechanism for EGFR Tyrosine Kinase Inhibitors-Associated Skin Toxicity?

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ABSTRACT

The dermatologic side effects are the most common adverse effects associated with Epidermal Growth Factor Receptor tyrosine kinase inhibitors. Although the mechanisms underlying the development of the skin toxicity remain unclear, immunological mechanisms are considered to be involved. A possible correlation between plasma levels of certain cytokines and development of skin toxicity has been reported. The aim of this work was to investigate the possible contribution of IL-31 and IL-33, cytokines involved in disorders associated with itching, in the pathogenesis of pruritus in patients undergoing EGFR-TK inhibitors. We report a significant increase of IL-31 and IL-33 serum levels in a patient with a bronchioalveolar carcinoma, who had showed previous skin rash, xerosis, and pruritus during treatment with different EGFR-TK inhibitors. She developed intense iching during gefitinib therapy. Therefore, we had collected patient blood sample to evaluate IL-31 and IL-33 serum levels compared to controls, reporting a significant increase in serum of patient. In the light of these findings, EGFR-TK inhibitors-related symptoms of dermatologic toxicities might be related to the release of IL-31 and IL-33. In particular, it is supposable that EGFR-TK inhibitors could cause keratinocytes injury, the release of IL-33 and the consequent interaction with its receptor on mast cells, that induces the secretion of several factors capable to cause skin manifestations, included IL-31, a known pruritus-inducing cytokine. This report, to the best of our knowledge, is the first work describing a possible involvement of IL-31/IL-33 axis in the pathogenesis of skin side effects related to EGFR-TK inhibitors treatment. J. Cell. Biochem. 114: 2673–2676, 2013.

KEY WORDS: INTERLEUKIN-31 (IL-31); INTERLEUKIN-33 (IL-33); EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS; PRURITUS; SKIN TOXICITY; CYTOKINES

The epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitors have been found overexpressed, in a variety of solid tumors, including non-small cell lung cancer [Franklin et al., 2002].

Tyrosine kinases play a critical role in the modulation of growth factor signaling. Once activated, these enzymes can cause increase in tumor cell proliferation and growth, induce antiapoptotic effects, and promote angiogenesis and metastasis. Therefore, pharmacological blockage of tyrosine kinase receptors prevent their activation and the multiple downstream signaling pathways involved in the cancer pathogenesis.

Epidermal growth factor receptor tyrosine kinase inhibitors are powerful weapons in the arsenal of cancer therapies against advanced and metastatic non-small-cell lung cancer. These treatments have a more favorable side effect profile than chemotherapy,

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with a distinct toxicity pattern, namely gastrointestinal and skin adverse events.

The dermatologic side effects of these drugs affect more than 80% of patients and are the most common adverse effects associated with EGFR inhibitors [Peuvrel et al., 2012].

Presently, the pathophysiology of skin toxicity remains unclear, nevertheless the involvement of immunological mechanisms has been hypothesized. Keratinocytes represent 95% of epidermal cells. Although the primary function of these cells is to provide the structural integrity and barrier function of the epidermis, it is now well accepted that they play an important role in the initiation and perpetuation of skin inflammatory, immunological reactions and the pathogenesis of inflammatory skin diseases [Miodovnik et al., 2012].

A recent study [Cirillo and Prime, 2011] has reported that keratinocytes produce and metabolize cortisol endogenously, representing a key determinant in inflammatory diseases.

Moreover, a possible correlation between plasma levels of certain cytokines and development of skin toxicity have been reported.

A study by Rodeck [2009] has revealed that EGFR-dependent control of IL-1 signaling in keratinocytes of the outer root sheath of the hair follicle constitutes an important pathogenic pathway to skin toxicity associated with the use of EGFR inhibitors.

IL-31 is a new member of the IL-6 family cytokines, mainly expressed in pruritic disorders [Cornelissen et al., 2012].

IL-33 is a recently recognized cytokine of the IL-1 cytokine family, that appears to drive T helper type 2 (Th2) responses. It can be secreted from damaged and inflamed tissues and seems to function as an alarmin in sensing damage in several inflammatory diseases, including atopic dermatitis (AD) [Pushparaj et al., 2009].

The aim of this work was to investigate the possible involvement of IL-31 and IL-33 in the skin side effects caused by EGFR inhibitors treatment.

CASE PRESENTATION

A 61-year-old non-smoking woman was referred to our institution in May 2008 for the presence of a bronchioalveolar carcinoma of the right lung with controlateral lung metastases, diagnosed after a transbronchial biopsy.

The patient was treated with three cycle of cisplatin plus pemetrexed, stopped because of disease progression, documented to computed tomography (CT) scan. No toxicity was reported.

Subsequently, a second line treatment with Erlotinib 150 mg/day orally was started in September 2008.

After 2 months, a remarkable improvement of the lung metastases was observed. The treatment was continued until November 2010, when a CT scan showed a disease progression.

During Erlotinib treatment grade 2 skin rash, xerosis, and related pruritus (NCI-CTCAE v4.0) were recorded, treated with symptomatic and topical drugs.

After skin toxicity resolution pruritus persisted for 2–3 weeks.

In view of her good clinical conditions a third line treatment was started with Gemcitabine 1,000 mg/mq 1, 8, 15 q28. After three cycles, a new CT scan was performed and a disease progression was reported.

Therefore, after written informed consent, she was enrolled in a experimental protocol and an irreversible EGFR inhibitor was administered for 3 months.

She obtained a stable disease, but after the appearance of grade 3 skin rash, xerosis, and related grade 2 pruritus, the treatment was discontinued due to the persistence of sympthoms more than 3 weeks, in particular pruritus.

Three months later, CT scan demonstrated a disease progression.

On the basis of the previous sensitivity to EGFR TKI inhibitors, with a significant clinical benefit and a long standing response to erlotinib, the relatively refractory exhibited to chemotherapy agents and the good performance status, a rebiopsy was performed to assess EGFR status.

Molecular analysis showed an EGFR deletion in exon 19 and treatment with gefitinib was started in January 2012. After 1 month of therapy, the patient developed a G2 xerosis and pruritus.

METHODS AND RESULTS

We evaluated IL-31 and IL-33 serum levels in this patient and 10 sex and age matched healthy controls.

After obtaining written informed consent, a 10-ml blood sample was collected from the antecubital vein, to performed IL-31 and IL-33 serum levels.

IL-31 and IL-33 protein levels were measured using the commercially available DuoSet ELISA Development System kits (R&D Systems; Minneapolis, MN) specific for human IL-31 and human IL-33, respectively.

All analyses were performed according to the manufacturer's protocol.

IL-31 and IL-33 serum levels were significantly higher than in the control group (respectively 5,725.9 pg/ml vs. 1,722.9 pg/ml and 1,593.5 pg/ml vs. 785 pg/ml; Table I).

DISCUSSION AND CONCLUSIONS

Tyrosine kinase inhibitors are small molecules given orally that target the EGFR receptor. By blocking the intracellular ATP binding site,

TABLE I. Serum IL-31 and IL-33 Levels of Controls and Patient

Control N	IL-31 serum levels (pg/ml)	IL-33 serum levels (pg/ml)
1	157.6	117.3
2	112.0	29.4
3	237.8	159.2
4	1,641.1	785.0
5	881.6	267.1
6	278.5	118.6
7	1,722.9	665.1
8	83.0	63.8
9	25.2	96.1
10	557.6	115.9
Mean	569,73	241,75
Standard deviation	639,68	263,79
Patient	5,725.9	1,593.5

they prevent TK phosphorylation, thereby inhibiting the signaling cascade that activates growth and proliferation factors.

Effects of EGFR inhibition include impaired growth and migration of keratinocytes, and inflammatory chemokines expression by these cells [Lacouture, 2006]. These effects cause inflammatory cell recruitment and subsequent cutaneous injury, which accounts for the majority of symptoms, including tenderness, papulopustular rash and hair and periungual alterations [Robert et al., 2005].

Indeed, it has been demonstrated that inhibition of EGFR activation by an anti-EGFR antagonistic monoclonal antibody, induces extensive death with several features of apoptosis in keratinocytes in culture [Rodeck et al., 1997; Nardone et al., 2010].

In this article, we have reported the case of a patient with a bronchioalveolar carcinoma, who has developed repeated grade 2 skin rash, xerosis, and pruritus during treatment with three different EGFR-TK inhibitors. In order to better understand the pathogenesis of pruritus in patients undergoing EGFR-TK inhibitors treatment, we detected the serum levels of IL-31 and IL-33, cytokines associated with itching diseases in this patient and in a small control group.

Inflamed skin pruritus is often caused by the release of histamine from dermal mast cells [Lacouture, 2006].

Recent evidences suggest that pruritus caused by the chronicity of xerosis could also be mediated by a periadnexial accumulation of mast cells with subsequent release of mast cell mediators that can directly activate sensory nerves [Gerber et al., 2010].

Interleukin (IL)-31 is a novel T helper (Th) 2 cytokine which is mainly produced by the CD45RO+ cutaneous lymphocyte antigen (CLA)+ T lymphocytes [Bilsborough et al., 2006].

It is involved in both innate and adaptive immunity in tissues that are in close contact with the external environment, that is, the skin, the airways and the lung, and the lining of the intestine [Cornelissen et al., 2012].

In particular, recently IL-31 has been demonstrated to be produced by human mast cells [Cornelissen et al., 2012]; in addition monocytes, macrophages, monocyte-derived dendritic cells produce IL-31 in response to UV irradiation and H_2O_2 treatment [Miodovnik et al., 2012]. Moreover, normal human epidermal keratinocytes and dermal fibroblast show enhanced IL-31 mRNA expression upon H_2O_2 stimulation [Cornelissen et al., 2011].

Enhanced expression of IL-31 is associated with a number of diseases, including pruritic diseases such as AD, allergic contact dermatitis (ACD), prurigo nodularis, chronic urticaria [Cornelissen et al., 2012]. In particular, skin biopsies of AD and prurigo nodularis patients express increased IL-31 mRNA levels, while psoriasis samples were comparable to healthy skin [Sonkoly et al., 2006].

AD and prurigo nodularis (as well as ACD) are dermatologic disorders frequently accompanied by severe itch while psoriasis rarely causes pruritus. This indicates a connection of IL-31 with pruritic skin diseases.

Genome-wide gene expression analysis in different human tissues revealed that dorsal root ganglia express the high levels of IL-31RA mRNA. Since the sensation of itch is mediated by unmyelinated C fibers of primary sensory neurons whose cell bodies reside in the dorsal root ganglia, IL-31 is likely to induce pruritus by directly modulating the function of sensory neurons [Sonkoly et al., 2006]. It is currently argued that IL-31 may cause pruritus through the induction of a yet unknown keratinocyte-derived mediator, which subsequently activates unmyelinated C fibers in the skin. This suggests that IL-31 represents possibly a new link between the immune and the nervous system [Steinhoff et al., 2006].

The link between pruritus and IL-31 has also been demonstrated by a study showing that transgenic mice models over-expressing IL-31 developed severe pruritus with alopecia and skin lesions as hyperkeratosis, acanthosis, inflammatory cell infiltration, and increase in mast cells [Dillon et al., 2004].

IL-33 has recently been attributed to the epithelial "alarmin" defense system. IL-33 is released by the epithelial cells in various tissues and organs, including keratinocytes, endothelial cells, and immune cells. In particular, IL-33, as other cytokines of IL-1 family, is released by necrotic structural cells, as fibroblasts and keratinocytes [Enoksson et al., 2011]. IL-33 exerts its effects by activating the ST2 (suppression of tumorigenicity 2)/IL-1 aR receptor on different types of cells, including mast cells and Th2 cells [Cevikbas and Steinhoff, 2012]. It has been recently demonstrated that IL-33 released upon injury by structural cells is recognized by T1/ST2 receptors on the surface of mast cells; this results in the secretion of proinflammatory factors, including IL-6, TNF-α and leukotrienes. Subsequently, these signals can induce vascular changes, including vasodilatation, increased permeability of the microvasculature and recruitment of inflammatory cells to the site of injury [Enoksson et al., 2011].

It could be hypothesized the secretion of IL-31 by mast cells after IL-33 stimulation.

In several organs, IL-33 appears to drive T helper type 2 (Th2) responses, suggesting a possible role in allergic and atopic diseases, as well as in fibrosis.

Recently, mounting evidence support the involvement of IL-31 and IL-33 in the pathogenesis of AD [Wong et al., 2012]. Moreover, the receptors of the two cytokines, IL-31RA and ST2 were found to be expressed on dermal fibroblasts and it is hypothesized that IL-31 and IL-33 could synergistically stimulate AD-related chemokines release from basophils interacting with fibroblasts [Wong et al., 2012].

In the light of these findings, it is presumable that the skin manifestations and itch caused by EGFR-TK inhibitors treatment could be related to the release of IL-31 and IL-33. In particular, it is supposable that EGFR-TK inhibitors can cause keratinocytes injury [Rodeck et al., 1997] with release of IL-33, which in turn interacts with its receptor on mast cells leading to the secretion of several factors capable to cause skin manifestations, included IL-31, a known pruritus-inducing cytokine.

The manifestations showed by our patient are clearly related to the EGFR-TK inhibitors therapy, since pruritus and skin rash appeared for three times only during treatment with these drugs and disappeared after therapy discontinuation. In this patient, we found very high serum levels of IL-31 and IL-33 compared to 10 healthy controls. Patient blood sample was collected during treatment with gefitinib, in presence of skin manifestations, therefore we had supposed an interesting correlation between cytokines levels and the skin reaction caused by EGFR-TK inhibitors treatment.

This report is, to the best of our knowledge, the first work that describe a possible involvement of IL-31 and IL-33 in the

pathogenesis of skin side effects related to EGFR-TK inhibitors treatment.

Further validations and investigations will be needed to better define the role of these cytokines in this setting.

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