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Interleukins as new prognostic genetic biomarkers in non-small cell lung cancer

Cristina Pérez-Ramírez [a, b], Marisa Cañadas-Garre [c, *], Ahmed Alnatsha [a, d], Miguel Ángel Molina [e], Ana I. Robles [f], Eduardo Villar [g], Juan Ramón Delgado [h], María José Faus-Dáder [b], Miguel Ángel Calleja-Hernández [i]

[a] Pharmacogenetics Unit, UGC Provincial de Farmacia de Granada, Instituto de Investigación Biosanitaria de Granada, Complejo Hospitalario Universitario de Granada, Avda. Fuerzas Armadas, 2, Spain
[b] Department of Biochemistry, Faculty of Pharmacy, University of Granada, Campus Universitario de Cartuja, s/n, 18071 Granada, Spain
[c] Centre for Public Health, Queen's University of Belfast c/o Regional Genetics Centre, Level A, Tower Block, Belfast City Hospital, Lisburn Road, Belfast, BT9 7AB, United Kingdom
[d] Department of Molecular Medicine, Faculty of Medicine, University of Tübingen, Geissweg 5, 72076 Tübingen, Germany
[e] Pangaea Biotech, S.L., Hospital Universitario Quirón Dexeus, C/ Sabino Arana, 5-19, 08028 Barcelona, Spain
[f] National Cancer Institute, 37 Convent Dr, 37/3060D, Bethesda, MD, United States
[g] Pathology Service, UGC Anatomía Patológica, Instituto de Investigación Biosanitaria de Granada, Complejo Hospitalario Universitario de Granada, Avda. Fuerzas Armadas, 2, 18014 Granada, Spain
[h] Medical Oncology Service, UGC Oncología Médica, Instituto de Investigación Biosanitaria de Granada, Complejo Hospitalario Universitario de Granada, Avda. Fuerzas Armadas, 2, 18014 Granada, Spain
[i] Department of Pharmacology, Faculty of Pharmacy, University of Granada, Campus Universitario de Cartuja, s/n, 18071 Granada, Spain

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ABSTRACT

Background: Surgery is the standard treatment for early-stage NSCLC, and platinum-based chemotherapy remains as the treatment of choice for advanced-stage NSCLC patients with naïve EGFR status. However, overall 5-years relative survival rates are low. Interleukins (ILs) are crucial for processes associated with tumor development. In NSCLC, *IL1B, IL6, IL12A, IL13* and *IL16* gene polymorphisms may contribute to individual variation in terms of patient survival. The purpose of this study was to evaluate the association between IL gene polymorphisms and survival in NSCLC patients.
Methods: A prospective cohorts study was performed, including 170 NSCLC patients (114 Stage IIIB-IV, 56 Stage I-IIIA). *IL1B* (C > T; rs1143634), *IL1B* (C > T; rs12621220), *IL1B* (C > G; rs1143623), *IL1B* (A > G; rs16944), *IL1B* (C > T; rs1143627), *IL6* (C > G; rs1800795), *IL12A* (C > T; rs662959), *IL13* (A > C; rs1881457) and *IL16* (G > T; rs7170924) gene polymorphisms were analyzed by PCR Real-Time.
Results: Patients with *IL16* rs7170924-GG genotype were in higher risk of death ($p = 0.0139$; $HR = 1.82$; $CI_{95\%} = 1.13–2.94$) Furthermore, carriers of the TT genotype for *IL12A* rs662959 presented higher risk of progression in the non-resected NSCLC patient subgroup ($p = 0.0412$; $HR = 4.49$; $CI_{95\%} = 1.06–18.99$). The rest of polymorphisms showed no effect of on outcomes.
Conclusions: Our results suggest that *IL16* rs7170924-GG and *IL12A* rs662959-TT genotypes predict higher risk of death and progression, respectively, in NSCLC patients. No influence of *IL1B* rs12621220, *IL1B* rs1143623, *IL1B* rs16944, *IL1B* rs1143627, *IL6* rs1800795, *IL13* rs1881457 on NSCLC clinical outcomes was found in our patients.

1. Introduction

Lung cancer is the leading cause of death from cancer worldwide, accounting for ≈27% of all cancer deaths. This type of cancer is the second most diagnosed in the United States (after prostate and breast cancer), with an incidence rate over 14% in both genders,

* Corresponding author.
E-mail addresses: cperezramirez87@gmail.com (C. Pérez-Ramírez), marisacgarre@gmail.com (M. Cañadas-Garre), r.j.b-man@hotmail.com (A. Alnatsha), mamolina@pangaeabiotech.com (M.Á. Molina), Ana_Robles@nih.gov (A.I. Robles), eduardovillar6@gmail.com (E. Villar), juanramondelgado@gmail.com (J.R. Delgado), mfaus@ugr.es (M.J. Faus-Dáder), mangel.calleja.sspa@juntadeandalucia.es (M.Á. Calleja-Hernández).

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Abbreviations list

AJCC	American Joint Committee on Cancer
CHUG	Complejo Hospitalario Universitario de Granada
CI	Confidence Interval
EGFR	Epidermal Growth Factor
HR	Hazards Ratio
ILs	Interleukins
NA	Not Available
NSCLC	Non-Small Cell Lung Cancer
OS	Overall Survival
PFS	Progression Free-Survival
SNPs	Single Nucleotide Polymorphisms

and 117920 and 106470 estimated new cases for 2016 in men and women, respectively [1]. In accordance with the latest cancer statistics, 158080 new cases and 224390 deaths are expected to occur in 2016 [1].

Small cell lung cancer and non-small cell lung cancer (NSCLC) are the two main types of lung cancer. NSCLC represents around 80–85% of all lung cancer cases and is classified in three subtypes: squamous cell carcinoma, adenocarcinoma and large cell carcinoma. According to the American Joint Committee on Cancer (AJCC), most patients with NSCLC present late-stage (IIIB-IV) at the time of diagnosis [2–4].

The standard treatment for early-stage NSCLC is surgery, which may be followed up by platinum-based chemotherapy in patients at high risk of recurrence. Platinum-based chemotherapy remains the treatment of choice for advanced-stage NSCLC. This treatment is given for EGFR (epidermal growth factor receptor) and ALK-rearranged (anaplastic lymphoma kinase) naïve patients and as second line in mutated EGFR patients [5]. Anti-microtubule agents (taxanes and vinca alkaloids), antifolate agents (pemetrexed), or pyrimidine antagonists (gemcitabine) are usually given in combination with cisplatin or carboplatin. In comparison with best supportive care, platinum based chemotherapy has reported more benefits in terms of survival (27.0 vs 10.3 weeks, respectively; $p < 0.001$) and symptom control [6,7]. However, the overall response rate (ORR) to platinum-based regimen is about 13–47.2% and only 16% of the patients are alive five years after diagnosis [8–24]. Therefore, new therapeutic approaches are urgently needed to improve PFS and OS in advanced stage NSCLC. Pathologic staging is an essential prognostic factor for NSCLC, but a significantly variability in progression and survival among patients with the same stage of disease have been reported, suggesting other factors may influence NSCLC prognosis [4,25]. Remarkably, genetic alterations, such as single nucleotide polymorphisms (SNPs), have showed to be related with inter-individual differences in recurrence and survival in NSCLC patients [26–29].

Inflammation is a physiological process induced by immune cell to fight infections and heal wounds. Nevertheless, long-standing inflammation secondary to chronic infection may produce a continuous tissue damage and cellular proliferation that results in metaplasia and dysplasia [30,31]. Therefore, there is a notable association between chronic inflammation, infection and early stage of neoplastic development. In fact, clinical and epidemiological studies have reported that 20% tumors are associated to chronic infection [32]. Interleukins (ILs) are a family of cytokines, which play an essential role on growth, differentiation, and activation of immune cells [33]. Based on above, ILs are crucial for processes associated with tumor development [34]. They act as autocrine and paracrine growth factors, promoting growth and inhibiting apoptosis at the site of inflammation [34]. Remarkably, recent studies have reported a strong effect of *IL1B, IL6, IL12A, IL13* and *IL16* gene polymorphisms on survival of NSCLC patients [26,27].

IL1B is a pro-inflammatory cytokine that plays a crucial role on inflammatory response, inducing expression of functional genes involved in inflammation [35]. IL1B may be produced by lung epithelial cells and genetic alteration in this gene has showed an important effect on NSCLC development and progression [36–42]. IL6 is a potent pleiotropic inflammatory cytokine that is secreted by lymphoid and non-lymphoid cells, and is involved in important steps of tumor development, such as proliferation, angiogenesis and apoptosis [43–47]. Interestingly, IL6 is expressed in tumor epithelial cells and polymorphisms in this gene have been associated with poor prognosis in NSCLC patients [26]. IL12A is a multifunctional cytokine generated by dendritic cells, macrophages, neutrophils, and human B-lymphoblastoid cells that regulates immune response and induces anti-angiogenesis activity [48]. Germline variations in IL12A decrease its anti-angiogenic effect, resulting in increasing cancer progression [27]. IL13 is a T-cell derived immunoregulatory cytokine produced by T and B cells, mast cells, basophils, natural killer and dendritic cells that exerts a critical function on allergic reactions, inducing immunoglobulin E secretion from activated human B cells [49]. Particularly in cancer, IL13 has reported to be connected with tumor invasion and metastasis by enhancing MAPK pathway [50]. Thus, polymorphisms in this gene may alter regulation of IL13 production, increasing tumor progression [27]. IL16 is a pro-angiogenic cytokine produced by peripheral blood mononuclear cells that modulate T cell growth [51]. Studies in several types of solid tumors and hematologic malignancies have reported a strong association between IL16 levels and cancer progression [51]. Therefore, polymorphisms in *IL16* gene that reduce angiogenesis process may be responsible for changes in prognosis of NSCLC patients [27].

Based on above, the identification of genetic variants in *ILs* may be essential to predict NSCLC clinical outcomes. To date, there are few studies on germline variations in IL genes and lung cancer survival. In this study, we aimed to evaluate the association between IL gene polymorphisms and survival in NSCLC patients. To determine the impact of treatment, we also performed a subgroup analysis according to surgical resection.

2. Material and methods

A prospective cohorts study was conducted.

2.1. Ethics statement

This study was performed under the approval of the Complejo Hospitalario Universitario de Granada (CHUG) Ethics and Research Committee and in accordance with the declaration of Helsinki. A written informed consent form was signed by the patients for blood sample collection and genotyping analysis. The identification of samples was based on non-patient codes.

2.2. Study population

This study included 170 NSCLC patients recruited in CHUG, Granada, Spain, diagnosed between 2003 and 2015 and followed up until February 2016. The inclusion criteria for the group of patients were age $\geq$18 years, histologically or cytologically confirmed diagnosis of NSCLC (stages I-IV), an Eastern Cooperative Oncology Group (ECOG) performance status $\leq$2, an adequate organ function, measurable disease by chest computed tomography scan, no previous treatment and available clinical data.