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Therapeutic potential of single-nucleotide polymorphism-mediated interleukin-6 receptor blockade in cancer treatment: A Mendelian randomization study

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ABSTRACT

Background: Interleukin-6 (IL-6) is a crucial member of the cytokine network and plays a pivotal role in the pathogenesis of various diseases, including cancer. IL-6 receptor (IL-6R) blockade is widely employed as a therapeutic strategy; however, its efficacy in anticancer therapy remains ambiguous.

Methods: An inverse variance-weighted Mendelian randomization (MR) analysis was conducted to assess the causal effects exerted by IL-6R blockade in remediating cancer. Drug-targeted single-nucleotide polymorphisms (SNPs) were introduced within 300 kb of the IL-6R gene. An instrumental variable comprising 26 SNPs represented IL-6 signaling downregulation and C-reactive protein level reduction. Datasets pertaining to the 33 types of cancer investigated in this study were acquired from the FinnGen genome-wide association study.

Results: The selected instrumental variable lowered fibrinogen levels, confirming its ability to mimic IL-6R blockade. IL-6R blockade exhibited therapeutic effects on five different cancer types documented in the FinnGen database (N = 334,364, including 76,781 cancer patients): bladder (odds ratios (OR) = 0.563), laryngeal (OR = 0.293), eye (OR = 0.098), gallbladder (OR = 0.059), and myeloid leukemia (OR = 0.442); however, it simultaneously elevated the risk of developing basal cell carcinoma (OR = 1.312) and melanoma (OR = 1.311). Sensitivity analyses did not alter the primary results.

Conclusion: Therefore, this study aimed to evaluate the potential and efficacy of SNP-based IL-6R blockade in treating cancer.

1. Introduction

Malignant tumors epitomize an aggressive, fatal disease that poses a crucial global health concern [1]. Statistics from the World Health Organization show that malignant tumors predominantly contribute to mortality rates, causing millions of fatalities annually.

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The incidence of malignancies continues to escalate, resulting in physical agony and financial burdens for patients and their families [2]. This gradual elevation in the prevalence of malignant tumors is attributable to alterations in human lifestyles and aging populations [3]. Currently, targeted therapy is a novel method for treating tumors. Contrary to conventional chemotherapy and radiotherapy, targeted therapy is designed based on the molecular characteristics of tumor cells; this enables precise targeting of cancer cells while minimizing damage to normal cells and the consequential risks and side effects [4].

Interleukin-6 (IL-6) is a pleiotropic cytokine that plays an pivotal role in the occurrence, development, and metastasis of tumors [5]. Presently, extant research indicates that IL-6 can potentially promote the growth and survival of tumors by stimulating tumor cell proliferation, inhibiting their apoptosis [6], and increasing the nutrient and oxygen supply to tumor cells through tumor-related neovascularization [7]. Furthermore, IL-6 has demonstrated the ability to enhance the invasive and metastatic efficiencies of cancer cells by regulating their interaction with the extracellular matrix [8]. Tocilizumab or sarilumab are IL-6 receptor antagonists that can inhibit membrane-bound and soluble IL-6R. This class of drugs has been used to treat various diseases, including COVID-19 [9], rheumatoid arthritis [10], juvenile idiopathic arthritis [11], and Castleman's disease [12]. As IL-6 plays an important role in the occurrence of tumors, it is currently of paramount importance to study whether IL-6R inhibitors that downregulate the IL-6 signaling pathway are useful for tumor treatment. Mendelian randomization (MR) is an experimental design that uses genetic variation in a population cohort as an instrumental variable to assess specific target causal effects, which can reduce the influence of external confounding factors on research results [13].

Therefore, this study aimed to employ a two-sample MR analysis to evaluate the effects of IL-6R inhibitors that downregulate the IL-6 signaling pathway on tumor treatment.

2. Material and methods

2.1. Source of outcome data

The electronic medical records on the genetic associations of 33 types of cancers, including 76,781 European patients diagnosed with cancer and 257,583 healthy European individuals without any cancer, were acquired in compliance with the FinnGen genome-wide association study (GWAS) Round 8 protocol. The cohort of cancer patients presented with basal cell carcinoma (16,328), melanoma (2,705), lymphoid leukemia (1,299), lung adenocarcinoma (1,237) and squamous cell carcinoma (1,210), multiple myeloma (1,085), meningeal carcinoma (1,043), follicular (955), diffuse large B-cell (780), Hodgkin (690), and mature T/NK-cell lymphomas (296), myeloid leukemia (582), carcinoma of the small intestine (455), glioblastoma (162), and breast (14,000), prostate (11,590), colorectal 5,458, cervical (2,913), bladder (2,380), kidney (1,830), endometrial (1,677), thyroid (1,525), ovarian (1,264), pancreatic (1,249), stomach (1,227), liver (648), esophageal (503), laryngeal (374), testicular (332), eye and adnexa (187), parotid gland (136), gallbladder (84), and small cell lung cancers (577).

2.2. Genetic instrument selection

In adherence to the established criteria for genome-wide significance ($p < 5 \times 10^{-8}$), minor allele frequency (>0.01), and exclusion of linkage disequilibrium ($r^2 > 0.1$), 26 single nucleotide polymorphisms (SNPs), which downregulate the IL-6 signaling pathway, were selected from an MR study that performed a meta-analysis of 522,681 European individuals from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium and UK Biobank (Supplementary Table 1) [14]. Linkage disequilibrium between the selected SNPs was estimated using the 1000 Genomes European Reference Panel [15]. These variants, located within a 300-kb region of the IL-6R gene and associated with high-sensitivity C-reactive protein (hsCRP), a downstream biomarker of the IL-6 signaling pathway, were assayed to monitor CRP levels. This genetic instrument mimics the effects of anti-IL-6R monoclonal antibodies by suppressing both the classical and trans signaling pathways of IL-6 [16]. Therefore, this genetic instrument was designated IL-6R blockade. Subsequently, the ability of the selected genetic variants to potentially mimic IL-6R blockade was demonstrated by evaluating the genetic association of IL-6R blockade with fibrinogen levels, which also serves as a downstream biomarker of the IL-6 signaling pathway.

2.3. Statistical analysis

An inverse variance-weighted (IVW) test using the TwoSampleMR package [17] in R software (version 4.2.1) (https://www.r-project.org/) was employed as the primary analytical method to estimate the association between IL-6R blockade and its anticancer therapeutic efficacy [13]. Additionally, we used other robust methods, such as MR-Egger [18], weighted median [19], simple mode [20], weighted mode [21], and MR-PRESSO [20], to consistently quantify the causal parameters without necessitating the validation of all genetic variants as instruments. The Cochrane's Q statistic derived from the IVW test was used to compute heterogeneity among estimated values for each SNP. An $F > 10$ prompted the assumption that the correlation was strong enough to avoid bias from weak instruments [22].

3. Results

3.1. Genetic association of 26-SNP instruments with IL-6 signaling pathway

This genetic instrument can reduce the expression level of IL-6 in both the classical and *trans*-signaling pathways (β: 0.631, p-value: 5.12E-09), particularly that of sIL-6R in the trans signaling pathway (β: 7.143, p-value: 1.68E-07) (Table 1). Furthermore, to confirm the impact of the 26-SNP instrument on the IL-6 signaling pathway, fibrinogen, a downstream target of the pathway, was selected as a biomarker. IL-6R blockade caused a decrease in fibrinogen levels (β: 0.328, weighted median; β: 0.500, weighted mode). This result did not exhibit any horizontal pleiotropy ($p > 0.05$, MR-Egger) (Fig. 1A and B).

3.2. Effect of IL-6R blockade on 33 types of cancer

An IVW test was selected as the primary analysis method to perform MR analysis on 33 types of cancer acquired from the FinnGen database owing to its efficacy in assessing causal associations and superior statistical power. As the instrument variable representing IL-6R blockade was weighted by CRP, odds ratios (ORs) were proportional to the natural logarithm of the decrease in CRP. IL-6R blockade had a therapeutic effect on five types of cancer, including myeloid leukemia (OR: 0.442, 95% CI: 0.205–0.952), bladder (OR: 0.563, 95% CI: 0.382–0.831), laryngeal (OR: 0.293, 95% CI: 0.114–0.754), eye (OR: 0.098, 95% CI: 0.025–0.381), and gallbladder cancers (OR: 0.059, 95% CI: 0.008–0.440). However, IL-6R blockade elevated the risk of basal cell carcinoma (OR: 1.312, 95% CI: 1.120–1.538) and melanoma (OR: 1.311, 95% CI: 1.111–1.546) (Figs. 2, 3A-G). Contrarily, IL-6R blockade exerted no notable effect on the remaining 26 types of cancer ($p > 0.05$) (Supplementary Figs. 1–4).

3.3. Effect of sIL-6R representing the IL-6 trans-signaling pathway on 33 cancers

In the IL-6 signaling pathway in tumors, IL-6 *trans*-signaling is thought to play a major role. Therefore, we chose rs2228145 as an instrumental variable for sIL-6R and analyzed the effect of the IL-6 *trans*-signaling pathway on 33 types of tumors [25]. The effect of sIL-6R on bladder cancer (OR: 1.083, 95% CI: 1.001–1.171), laryngeal cancer (OR: 1.270, 95% CI: 1.056–1.527), and cancer of the eye and adnexa (OR: 1.372, 95% CI: 1.042–1.806), implies that IL-6 receptor blockade may remediate these three cancers by suppressing trans signaling. Nonetheless, this causal relationship was not observed in other cancer types (Table 2 and Supplementary Table 2).

3.4. Sensitivity analysis

In the sensitivity analysis of the seven types of cancer affected by IL-6R blockade, no heterogeneity or pleiotropy was observed, with all p-values being greater than 0.05 (Table 3). In addition, leave-one-out analysis demonstrated that single SNP did not affect the outcome (Supplementary Fig. 5), and the funnel plots for the seven types of cancer were roughly symmetric (Supplementary Fig. 6). However, in the sensitivity analysis of the other 26 unaffected types of cancer, the IVW Cochrane Q statistic and MR-Egger detected mild to moderate heterogeneity in gastric and prostate cancers. In particular, the MR-PRESSO analysis of prostate cancer revealed an outlier (rs113580743), whose elimination stabilized the results (Supplementary Table 3). The integrated results depicted that no single SNP exerted any effect on the outcome (Supplementary Fig. 7), and the funnel plots were roughly symmetric (Supplementary Fig. 8).

4. Discussion

IL-6 has been repeatedly reported to play a critical role in inflammation-associated tumor and is associated with poor prognosis and elevated mortality in cancer patients [26]. IL-6 is one of the most common inflammatory factors that regulate various features of cancer, including promoting the growth and survival of pre-cancerous cells, stimulating angiogenesis, inhibiting apoptosis at the site of inflammation, promoting tumor progression, invasiveness, metastasis, and producing resistance to chemotherapy drugs [27–29]. Currently, the role of the IL-6/IL-6R signaling axis in the development, metastasis, and treatment resistance of cancer is very broad and complex. Studies have shown that the use of tocilizumab may be a strategy for treating chromosomal instability cancers that overexpress IL-6R [30,31].

In this study, we used GWAS data from 522,681 European individuals from CHARGE consortium and the UK Biobank to select a set

Table 1
The extent to which IL-6 receptor blockers suppress the signaling pathways.

Outcome	Sample	Method	Beta	Se	p-value
IL-6	Ahola-Olli 2017 [23]	IVW	−0.631	0.108	5.12E-09
		WM	−0.623	0.155	5.84E-05
		MR Egger	−0.347	0.226	0.141
sIL-6R	IMPROVE [24]	IVW	−7.143	1.365	1.68E-07
		WM	−8.415	1.126	7.72E-14
		MR Egger	−7.840	2.741	0.016

IVW, inverse variance weighted; WM, Weighted median; SE, standard error.

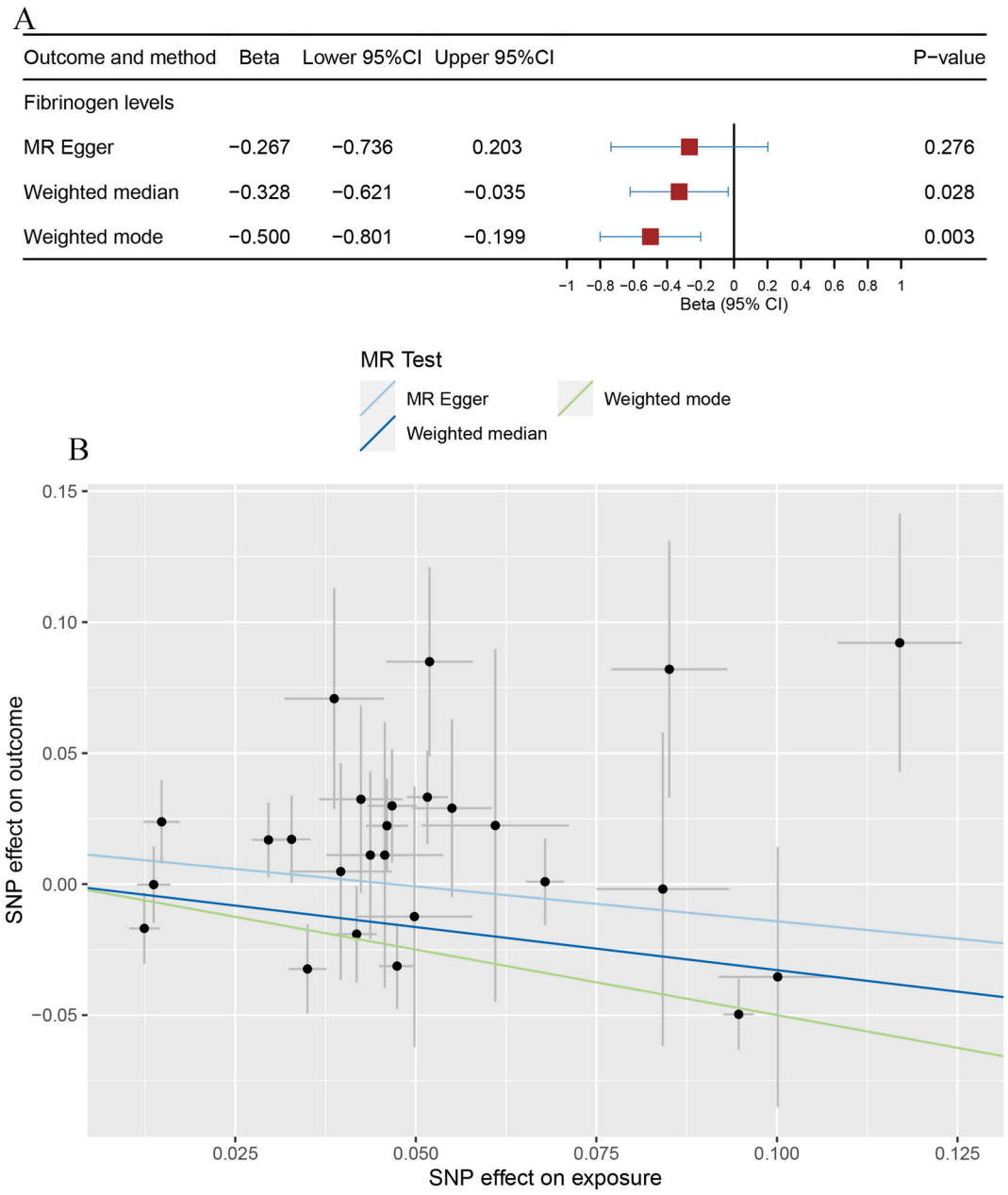

Fig. 1. Validation of the IL-6 signaling pathway on the downstream biomarker fibrinogen using three MR methods. (A) The results are presented in a forest plot and (B) a scatter plot. SNP effects were plotted into lines for MR-Egger regression (light blue line), weighted median estimator (dark blue line) and weighted mode (green line). The slope of the line corresponded to the causal estimation. CI, confidence interval.

of genetic instruments that could mimic the two modes of action exhibited by IL-6R blockers in impeding the IL-6 signaling pathways. The set contains 26 SNPs, with the smallest F statistic of $25 > 10$, indicating that our estimate of causal association is not affected by weak instrument bias. To avoid overlap with the European population screened for exposure, we selected 34 European cancer types from the FinnGen database as outcome variables. In the multiplicity testing analysis, we found that there was horizontal pleiotropy for the MR analysis of IL-6R blockers in 119 renal pelvic cancer patients. Therefore, we excluded renal pelvic cancer from the database, which included a total of 33 types of cancer. At the same time, we found heterogeneity in the outcome caused by rs113580743 in the MR-PRESSO analysis of IL-6R blockers on prostate cancer. After excluding this SNP, we reanalyzed the data and discovered that IL-6R blockers neither possess therapeutic effects nor increase the risk of developing prostate cancer.

Currently, phase II multicenter studies reveal that siltuximab, which also downregulates the IL-6 signaling pathway, lacks efficacy in patients with late-stage multiple myeloma [32,33]. Additionally, consistent with our results, tocilizumab inhibits IL-6R to overcome chemotherapy resistance induced by head and neck cancer stem cells in an animal experiment and has an anti-proliferative effect,

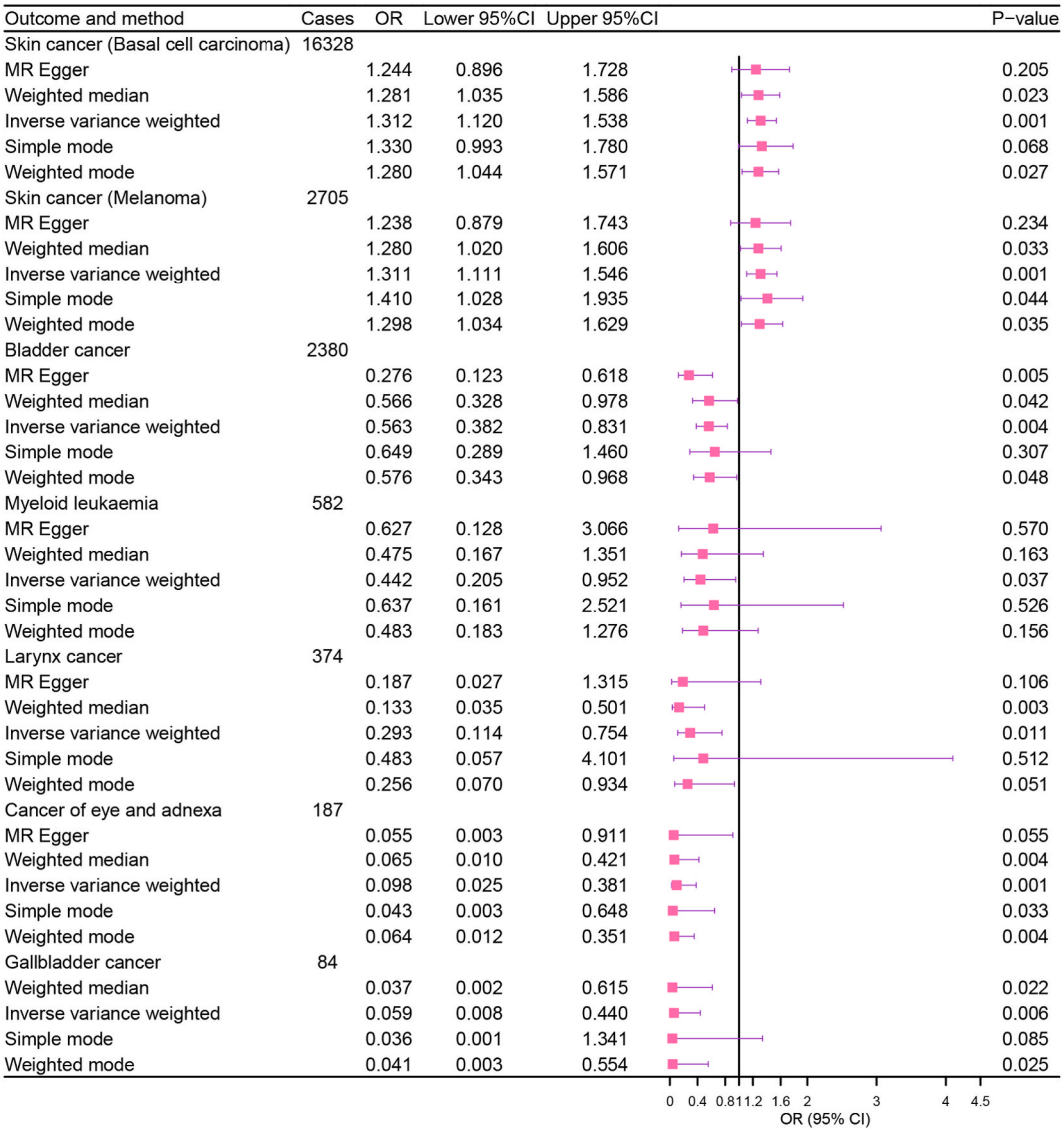

Fig. 2. Forest plot for estimating the causal effect of IL-6 receptor blockade on seven cancers using five MR methods, with inverse variance weighted as the primary MR analysis method. OR, odds ratio; CI, confidence interval.

which may be a potential treatment for head and neck squamous cell carcinoma [34]. However, a study found that knocking down tocilizumab and MCT-1 synergistically inhibits the stemness of triple-negative breast cancer in an animal experiment [35]. In addition, a study found that tumor-associated macrophages produce IL-6 to promote the expansion of human hepatocellular carcinoma stem cells in vitro [36], which unfortunately was not found in our results. Additionally, it has been reported that treatment of rheumatoid arthritis patients with tocilizumab may lead to complications such as infections and malignant tumors. Long-term use of tocilizumab may also result in malignant tumors such as progressive nodular melanoma [37,38]. The dual nature of IL-6R blockade has also been verified in our research. In our MR analysis, IL-6R blockade may be effective in treating five types of cancer, including bladder cancer, myeloid leukemia, laryngeal cancer, eye cancer, and gallbladder cancer. The effectiveness of treating laryngeal cancer has already been confirmed at the animal level [34]. Several clinical studies have confirmed that IL-6R blockade may increase the risk of two types of cancer, basal cell carcinoma and melanoma [37,39,40].

With the deepening understanding of IL-6 biology, two different modes of IL-6-mediated signaling, IL-6 classic and *trans*-signaling, have been widely recognized. Canonical IL-6 signaling involves the binding of IL-6 to the membrane-bound IL-6 receptor α subunit and the glycoprotein 130 (gp130) signaling subunit, and this membrane-bound IL-6R-mediated IL-6 activity is protective and regenerative. In contrast, in IL-6 trans signaling, a complex of IL-6 and the soluble form of the IL-6 receptor (sIL-6R) signals through membrane-bound gp130, which is the pro-inflammatory activity of the cytokine [41]. Even so, the involvement of IL-6 in cancer cell proliferation remains controversial in general, and the reasons for its complexity remain unclear [42]. As in melanoma, the role of IL-6 is quite

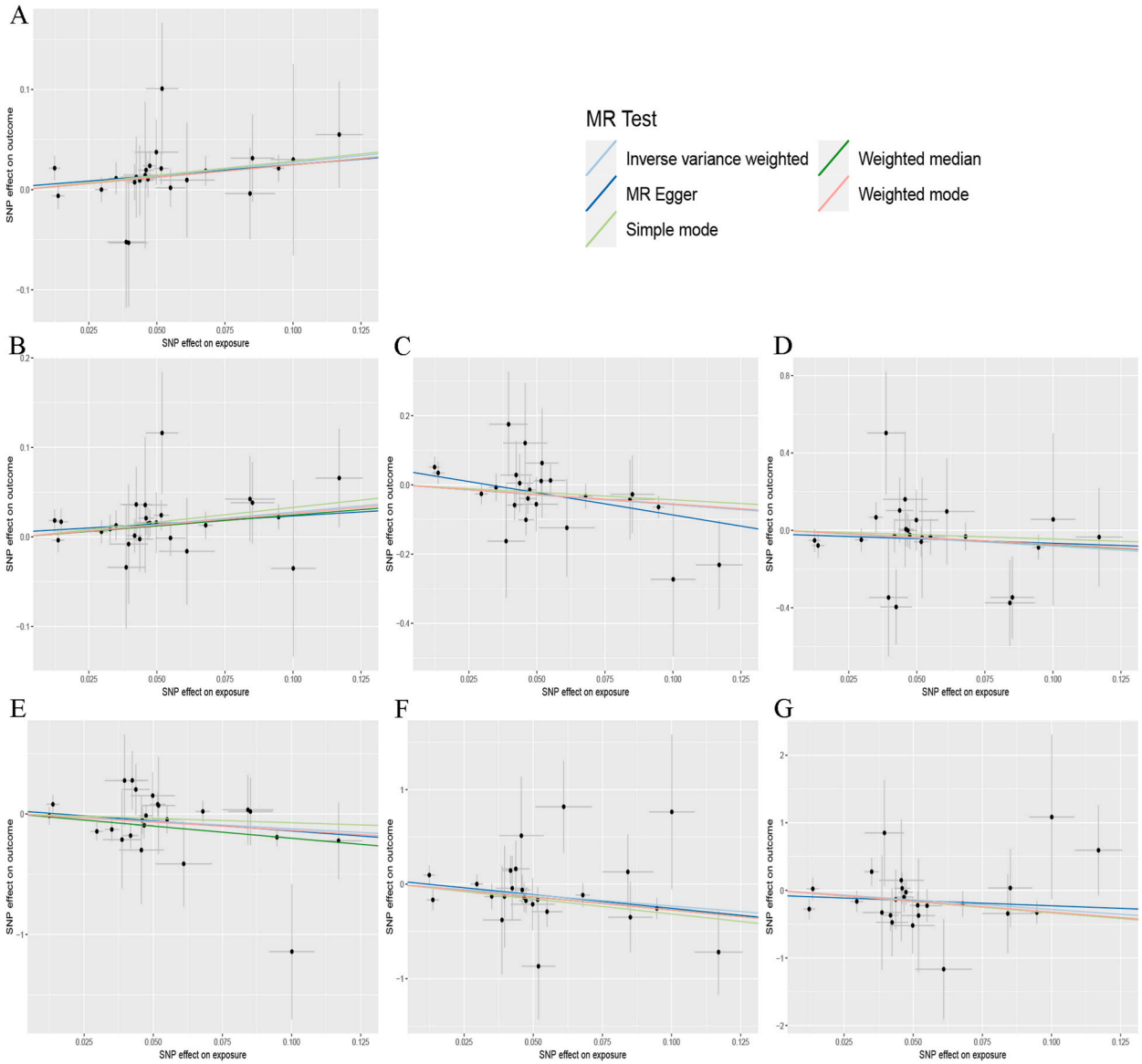

Fig. 3. Scatter plots of the 5 MR analysis methods. The causal effect of IL-6 receptor blockade on (A) basal cell carcinoma, (B) melanoma, (C) bladder cancer, (D) myeloid leukemia, (E) laryngeal cancer, (F) eye cancer, and (G) gallbladder cancer.

Table 2
Analysis of the effect of sIL-6R (rs2228145)-mediated trans signaling on seven tumor types by IVW method.

Outcome	OR	Lower 95%CI	Upper 95%CI	p-value
Skin cancer (Basal cell carcinoma)	0.974	0.943	1.006	0.108
Skin cancer (Melanoma)	1.012	0.941	1.089	0.744
Bladder cancer	1.083	1.001	1.171	0.048
Myeloid leukemia	1.115	0.954	1.302	0.170
Larynx cancer	1.270	1.056	1.527	0.011
Cancer of eye and adnexa	1.372	1.042	1.806	0.024
Gallbladder cancer	1.500	0.997	2.256	0.052

OR, odds ratio; CI, confidence interval.

complex, as it acts as a growth inhibitor in the early stage and as a growth factor in metastatic disease [43–45]. Thus, the use of tocilizumab increases the risk of this disease. We have been using antibodies that block overall IL-6 signaling for over a decade. However, there will always be some people who experience treatment side effects, such as IBD [46]. Today, IL-6 *trans*-signaling is

Table 3
Sensitivity analysis of IL-6 receptor blockade affecting seven tumor types.

Outcome	Heterogeneity			Pleiotropy		
	IVW Q (p-value)	MR-Egger Q (p-value)	MR-PRESSO RSSobs (p-value)	MR Egger's intercept	SE	p-value
Skin cancer (Basal cell carcinoma)	9.487 (0.994)	9.355 (0.991)	9.946 (0.996)	0.003	0.009	0.720
Skin cancer (Melanoma)	8.170 (0.998)	8.030 (0.997)	29.144 (0.258)	0.003	0.009	0.712
Bladder cancer	18.427 (0.734)	14.493 (0.883)	19.387 (0.774)	0.042	0.021	0.060
Myeloid leukemia	16.773 (0.820)	16.531 (0.789)	17.313 (0.863)	−0.020	0.041	0.628
Larynx cancer	21.349 (0.560)	21.082 (0.516)	24.158 (0.510)	0.027	0.051	0.611
Cancer of eye and adnexa	18.810 (0.712)	18.597 (0.670)	19.653 (0.752)	0.034	0.073	0.649
Gallbladder cancer	16.778 (0.820)	16.295 (0.801)	18.660 (0.880)	−0.076	0.109	0.494

IVW, inverse variance weighted; MR, Mendelian randomization; MR-PRESSO, MR pleiotropy residual sum and outlier; RSSobs, observed residual sum of squares; SE, standard error.

recognized as a biologically important independent pathway of cytokine receptor activation, and it has also become a pathological model of IL-6 signaling in several malignancies, such as colon cancer, liver cancer, and non-small cell lung cancer [47]. The first selective IL-6 *trans*-signaling inhibitor, olamkicept, has also achieved remarkable results in phase II clinical studies for the treatment of IBD [48]. Therefore, we analyzed the effect of sIL-6R, which represents the IL-6 *trans*-signaling pathway, as a genetic tool in cancer. Interestingly, we found a positive causal relationship between sIL-6R expression and the risk of bladder, larynx, eye, and adnexal cancers. This may provide signaling pathway support for IL-6 receptor blockers to treat these three cancers.

There are also many limitations in our study. First, MR is a method that uses genetic variation to estimate causality between exposure and outcome. Our genetic instruments only mimic the effects of anti-IL-6R monoclonal antibodies (such as tocilizumab). The inhibitory effect of this genetic instrument on the IL-6 signaling pathway is significantly lower than that of therapeutic IL-6R monoclonal antibodies [16,49,50]. Second, in the outcome phenotype, most of the 33 types of cancer lack pathological classification, which may cause significant bias in the experimental results because different subtypes of the same cancer can have a high degree of heterogeneity. Third, the MR results we obtained are based on lifelong blockade of the IL-6 signaling pathway, rather than the short-term therapeutic effects of IL-6R monoclonal antibodies. Fourth, this study only can analyse genetic sIL6R but not soluble IL6R produced by ADAM17 or ADAM10 mediated shedding. This big part of IL6R trans signaling is and can not be covered by this study.

Overall, this study provides supportive data for the use of IL-6R blockade in the treatment of bladder cancer, myeloid leukemia, laryngeal cancer, eye cancer, and gallbladder cancer through MR analysis. Additionally, IL-6R blockade, through tocilizumab, has a positive effect on treating various immune-related diseases clinically. However, based on the data and clinical reality presented in this study, the prescription of such drugs should be more cautious, and attention should be paid to the risk of patients developing skin cancer.

Ethics statement

Review and/or approval by an ethics committee was not needed for this study because this study used published open data.

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Author contribution statement

Shuwan Zhang: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Wenchuan Zhang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Hanxue Sun, Rui Xue: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Qingjie Lv: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20474.

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