Combination of G-CSF Improves Radiation Response and Generate Abscopal Effects in Patients With NSCLC

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Research

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Abstract

Backgrounds:

Local radiation could enhance immune response that mediates tumor regression. Recent studies focus on adding immunotherapy to RT to improve treatment response and induce abscopal response. As a stimulator for granulocytes, the clinical anticancer effects of granulocyte-colony stimulating factor (G-CSF) are still controversial. In this study, we evaluated the synergistic effect of radiotherapy with G-CSF in patients with NSCLC, using a propensity-score (PS) matching method.

Results

After matching, 333 patients in the G-CSF group and 333 patients in the observation group were included. With a median follow-up of 18.4 months, combination with G-CSF significantly improved PFS (median, 12.1ms vs. 7.3ms, $p<.001$). Multivariate analysis revealed that N stage, M stage, BED and administration of G-CSF were independent factors for PFS. Combination with G-CSF significantly improves abscopal response of radiotherapy (26.8% vs. 14.8% $p=.001$). Synchronous chemotherapy showed a tendency toward a favorable indicator for abscopal response, but the difference did not reach statistical significance ($p=.089$).

Methods

From 2000 to 2018, 1312 patients with NSCLC received definite or palliative radiotherapy were included, of whom 468 (35.7%) patients received G-CSF during radiotherapy. Patients in the G-CSF group were matched in a 1:1 ratio according to their PS to the observation group with a caliper of 0.01.

Conclusion

Concurrent administration of G-CSF improved radiation response significantly and generated abscopal effects in NSCLC. It indicated that the combination of radiotherapy and G-CSF is potential a new strategy and should be further evaluated in prospective clinical trials.

1 Introduction

Recent studies have shown that local radiation could stimulate a systemic immune response. Radiation of tumor cells results in the release of tumor associated antigens and can also enhance the presentation of tumor associated antigens in draining lymph nodes, which leads to increased tumor recognition and antitumor activities(1–5). An abscopal response describes tumor regression at sites distant from the irradiated field, which is potentially mediated by the immune response stimulated by RT. It is a rare event in patients with metastatic diseases receiving palliative radiotherapy to a single metastasis(6). With the

development of immunotherapy in cancer treatment, recent studies focus on adding immunotherapy to RT to improve treatment response and induce abscopal response(7).

Neutrophils are extensively involved in autoimmune and infectious diseases. Accumulating data also suggests the important function of neutrophils in tumor progression by their interaction with tumor and immune cells (8, 9). Tumor infiltrating neutrophils are termed tumor associated neutrophils (TANs). TANs can have both pro-and anti-tumor effects (10–12). TANs affect tumor progression through various mechanisms, including tumor cell proliferation, supporting angiogenesis, and interaction with immune cells(8, 13). Numerous meta-analysis and retrospective studies have investigated the prognostic value of neutrophil counts, but the conclusions are debatable (14–17). Considering the importance and diversity functions of TANs in tumor immunology, targeting neutrophils could be a potential therapeutic strategy to combine with radiotherapy(18).

Granulocyte-colony stimulating factor (G-CSF) is a cytokine that stimulates the bone marrow to produce granulocytes and stem cells and release them into the blood. G-CSF also stimulates the survival, proliferation, differentiation and maturation of neutrophils (19–21). Recombinant human G-CSF (rhG-CSF) has been widely used to reduce the degree and duration of neutropenia induced by chemotherapy (22–28). Since the role of neutrophils in tumor immunology is still not fully understood, the clinical anticancer effects of G-CSF are controversial. A number of preclinical studies have found that overexpression of G-CSF induced the expansion of circulating pro-tumor neutrophils and promoted metastasis (29–31). On the contrary, a recent study in mice demonstrated that the concurrent administration of G-CSF enhanced RT-mediated antitumor activity by activating anti-tumor neutrophils (32). Furthermore, given rhG-CSF during radiotherapy has been suggested associated with survival benefit in several small sample retrospective studies (33). Herein, to test whether local radiotherapy and combination of G-CSF could improve radiation response and induce abscopal effects or not, we retrospectively analyzed 1312 patients with NSCLC with the use of propensity score (PS) matching method to eliminate selection biases as much as possible.

2 Methods

2.1 Patients

A total of 3112 consecutive patients with NSCLC diagnosed in Shandong Cancer Hospital from January 2000 through December 2018 were evaluated. Among these patients, 1201 patients with no radiotherapy, 174 patients without reliable histopathological results, 39 with prior carcinoma over the past 5 years, 110 patients received immunotherapy, and 276 patients with incomplete information and loss to follow-up after radiation were excluded. A total of 1312 patients who received radiotherapy with NSCLC were enrolled in the study. All patients were diagnosed based on typical clinical indicators and histopathological results. Clinical T, N, and M stage were diagnosed according to the NSCLC diagnostic criteria in the 7th edition of AJCC. The study has been reviewed and approved by the Ethics Committee of

Shandong Cancer Hospital and Institute, China. Either a signed informed consent or authorization from the National Supervisory for Welfare and Health was obtained for all patients.

2.2 Treatment strategy

All patients were administered radiotherapy, with some receiving or not receiving concurrent systemic therapy. All radiotherapy was performed with 3D-CRT or IMRT. According to the sites of radiation, 640 patients were coded for having received thoracic radiotherapy, 346 patients having craniocerebral radiotherapy, 250 patients having bone radiotherapy and 76 patients having other parenchyma metastases radiotherapy. The median radiation dose was 50 Gy. Because of different radiation fractionations adopted clinically, we use biological effective dose (BED) to estimate the biological effects of radiation. Synchronous chemotherapy (within 2 weeks before the start of radiotherapy or within 2 weeks after the finish of radiotherapy) was given to 381 (29%) patients.

2.3 Administration of rhG-CSF

For all patients, administration of rhG-CSF during radiotherapy, within 2 weeks before the start of radiotherapy or within 2 weeks after the finish of radiotherapy were defined as rhG-CSF received during radiotherapy. rhG-CSF (100 µg, Qilu Pharmaceutical Co., Ltd.) was given daily, via subcutaneous injection for the prevention and treatment of neutropenia caused by chemotherapy or radiotherapy. The median duration of rhG-CSF injection was 2 days (range from 1 to 8).

2.4 Outcomes

The primary endpoint was the progression free survival (PFS). The secondary endpoint was the proportion of patients with an abscopal response. Also, the predictive value of neutrophils and other clinicopathological characteristics were analyzed in the study.

PFS was defined from the first date of radiotherapy to the time of progression or last follow-up. An abscopal response was defined as at least a 30% decrease in the longest diameter of any measurable (≥ 1 cm) non-irradiated lesions from baseline (34). Treatment response was evaluated at 2-3 months after completion of radiotherapy. The absolute neutrophil count was obtained from the baseline whole blood count.

2.5 Statistical analysis

PS matching method was used to balance the distribution of baseline clinicopathological variables and evaluate the exact role of G-CSF in combination with radiotherapy between the G-CSF group and observation group. The propensity score was calculated using a logistic regression model based on 11 preoperative factors (age at diagnosis, gender, Karnofsky performance status (KPS) score, smoking history, pathological type, clinical T, N, and M stage, radiation sites, radiation technique, and synchronous chemotherapy) that were thought to be potentially associated with usage of G-CSF. Patients in G-CSF group were matched in a 1:1 ratio according to their PS to the observation group with a caliper of 0.01, using the nearest neighbor algorithm. (Fig. 1).

The clinicopathological variables were compared between groups by chi-square test and Kruskal-Wallis test. Survival curves were estimated by the Kaplan-Meier method and compared between groups by log-rank test. A Multivariate model was performed using the Cox proportional hazards algorithm with significant variables in univariate analysis. All analyses were performed using IBM SPSS Statistics 22 (IBM Corp., New York, NY, USA), with $p < 0.05$ considered to indicate significance.

3 Results

3.1 Characteristics before and after matching

In all, 1312 patients were enrolled, of whom 468 patients who received G-CSF were matched with 844 patients observed in a 1:1 ratio according to the calculated propensity score. Patients who matched above a caliper of 0.01 were excluded, and the remaining 333 patients in the G-CSF group and 333 patients in the observation group were included in this analysis (Table 1).

Table 1
Patient characteristics before and after propensity-score matching

Variables	Before PSM, No.(%)			After PSM, No.(%)		
	G-CSF (n=468)	NO G-CSF (n=844)	<i>P value</i>	G-CSF (n=333)	NO G-CSF (n=333)	SD
Age(years)						
≥60	208	408	.184	146	143	.018
<60	260	436		187	190	
Gender						
Male	323	591	.707	235	232	-.019
Female	145	253		98	101	
Smoking history						
Yes	343	610	.693	247	259	-.036
None	125	234		86	74	
KPS score						
≥90	165	314	.510	122	118	-.025
<90	303	530		211	215	
pathological type						
Squamous cell	170	233	.001	111	107	.025
Adenocarcinoma	298	621		222	226	
Tstage						
0-2	195	378	.296	141	133	.049
3-4	273	466		192	200	
Nstage						
N-	54	122	.151	32	31	.009
N+	414	722		301	302	
Mstage						
M0	158	230	.048	100	88	.076
M1	310	614		233	235	

	Before PSM, No.(%)			After PSM, No.(%)		
RT site						
Thoracic	272	368	.000	185	173	.074
Craniocerebral	105	241		76	83	-.051
Bone	70	180		60	69	.060
Other parenchyma metastases	21	55		12	8	.066
RT technique						
3DCRT	243	435	.908	170	169	.036
IMRT	225	409		163	164	
Synchronous chemotherapy						
Yes	220	161	.000	110	115	-.030
No	248	683		223	218	

Table 2
Univariate and multivariate analysis of prognosis for progression free survival

	Univariate			Multivariate		
	HR	95%CI	P value	HR	95%CI	P value
Age	.952	.786-1.154	.619			
Gender	.929	.755-1.144	.490			
KPS score	.783	.639-.961	.019	.874	.707-1.080	.212
Smoking index	1.064	.880-1.287	.523			
Pathological type	.761	.623-.931	.008	.853	.688-1.057	.146
cT	1.072	.883-1.302	.483			
cN	1.599	1.075-2.379	.020	1.582	1.062-2.357	.024
cM	1.742	1.397-2.173	.000	1.501	1.177-1.915	.001
RT technique	1.138	.940-1.377	.186			
Synchronous chemotherapy	1.015	.832-1.239	.881			
BED(>60Gy)	.672	.556-.813	.000	.850	.695-1.040	.114
G-CSF	.547	.452-.662	.000	.614	.460-.821	.001
Neutrophils (≥ 2.28)	1.637	1.353-1.980	.000	1.124	.841-1.502	.430

After matching, no significant difference between the G-CSF and observation groups was found based on age, gender, smoking history, KPS score, pathological type, cT, cN, cM, RT site, RT technique, and synchronous chemotherapy (all SD absolute values < 0.1).

3.2 RT combined with G-CSF significantly improve PFS

Patients were followed for a median of 18.4 months (range, 4.7-96.2 months). Kaplan-Meier curves of PFS according to the usage of G-CSF are shown in Figure 2. The PFS was significantly longer in patients with G-CSF than those in observed patients (median, 12.1 months vs. 6.8 months, $p < .001$).

In the matched population ($n=666$), 333 patients received G-CSF and 333 patients did not. Figure 3 shows the Kaplan-Meier curves of PFS between G-CSF and observation groups. Combination with G-CSF significantly improved PFS (median, 12.0 months vs. 7.2 months, $p = .001$). The PFS rates at 1 year of the G-CSF group and observation group were 34.2% and 14.7%, respectively.

The significance of prognosis with PFS was assessed based on the disease characteristics and characteristics of patients. Our univariate analysis demonstrated that KPS score ≥ 90 , squamous carcinoma, lymph node negative, M0, BED>60Gy, administration of G-CSF, and lower neutrophils before radiotherapy were associated with better PFS. Multivariate analysis revealed that N stage, M stage, BED and administration of G-CSF were independent factors for PFS.

3.3 Abscopal response

478 patients (235 G-CSF vs. 243 observed) with metastatic disease were included in the evaluation of abscopal response. 63 patients in the G-CSF group and 36 patients in the observation group achieved an abscopal response. Combination with G-CSF significantly improved abscopal response of radiotherapy (26.8% vs. 14.8% $p=.001$). Synchronous chemotherapy showed a tendency toward a favorable indicator for abscopal response, but the difference did not reach statistical significance ($p=.089$). BED>60Gy, radiation site, fractionation regimen, radiation intention and other factors showed no significant difference between abscopal group and non-abscopal response. (Table 3)

Table 3
Clinical characteristics between abscopal group and non-abscopal response

	Abscopal response (n=99)	Non-abscopal response (n=379)	P value
Age(years)			.755
≥60	38	152	
<60	61	227	
Gender			.701
Male	63	249	
Female	36	130	
Smoking history			.280
Yes	41	180	
None	58	199	
KPS score			.790
>80	33	121	
≤80	66	258	
pathological type			.874
Squamous cell	24	89	
Adenocarcinoma	75	290	
RT site			.018
Thoracic	38	142	
Craniocerebral	28	127	
Bone	24	102	
Other parenchyma metastases	9	8	
RT technique			.981
3DCRT	50	278	
IMRT	49	271	
Synchronous chemotherapy			.086
Yes	44	133	
No	55	246	

	Abscopal response (n=99)	Non-abscopal response (n=379)	P value
BED			.284
<60	55	233	
>60	44	146	
G-CSF			.001
Yes	63	172	
No	36	207	
Fractionation			.815
Hypofractionation	16	65	
Conventional fractionation	83	314	
RT intention			.108
Curative	65	276	
Palliative	34	98	

4 Discussions

Our study demonstrated the promising effects of G-CSF combined with radiation by analyzing 1312 patients with NSCLC. Because this study is not a randomized study, the propensity score (PS) matching method was used to eliminate selection biases. We found that concurrent administration of rhG-CSF improved radiation response significantly and generated abscopal effects in NSCLC.

Currently, rhG-CSF is widely used in clinical to stimulate the production of neutrophils in patients who suffer from chemotherapy or radiotherapy-induced neutropenia. However, the anticancer effects of G-CSF are still unclear. Several studies have demonstrated the promising effects of G-CSF with chemotherapy for acute myeloid leukemia (35, 36). The underline mechanisms were that G-CSF drove leukemic cells into the cell cycle, which was more sensitive to chemotherapy. However, rare clinical evidence showed for solid tumors. A study that included 7 patients indicated the synergistic effect of G-CSF and chemotherapy for metastatic melanoma (37). On the contrary, some researchers have described the potential adverse effects of G-CSF (38–40). Shojaei et al. found that G-CSF mediated tumor refractoriness to anti-VEGF therapy by stimulating myeloid cell mobilization and angiogenesis in mouse models (41). Another preclinical study also found that G-CSF diminished the antitumor activity of chemotherapy by contributing to tumor revascularization (38). These controversial results may be related to that G-CSF can increase or activate the activity and recruitment of not only antitumor TANs but also protumor TANs and other myeloid-derived suppressor cells (MDSCs)(42). In addition, different types of neutrophils and other MDSCs have variable effects depending on the therapeutic regimen used and tumor growth stage.

Recent studies have revealed that radiation could enhance immune response that mediates tumor regression (43–45), but the immunologic effects of radiation has not been fully explored. TANs play important roles in tumor immunity and tumor development. Traditionally, TANs were mostly described for their ability to promote tumor progression through different mechanisms, including accessing tumor cell proliferation, suppressing the function of CD8+T cells (8, 46). Recently, accumulating data suggested that TANs also play roles in antitumor immunity. TANs could induce tumor cell apoptosis by secreting cytotoxic molecules such as ROS (32), or promoting the detachment of tumor cells leading to tumor cell death (32). Therefore, as the main stimulator of neutrophils, G-CSF had reversible effects when combined with radiation. Takeshima et al observed that radiation recruited tumor-associated neutrophils (RT-Ns) and induced apoptosis of tumor cells by increasing the production of reactive oxygen species. Also, they found the concurrent administration of G-CSF enhanced RT-mediated antitumor activity by activating RT-Ns (32). However, another study conducted by Kim et al. found that administration of G-CSF with radiotherapy promoted tumor growth by stimulating vascularization in tumor-bearing mice (47). Most of these data stay at the preclinical level. As to our knowledge, our study was first to demonstrate the synergistic effect of G-CSF to combine with radiation in patients with NSCLC. G-CSF significantly promoted radiation response and increased abscopal effects. In our results, low baseline neutrophils (<3.59) were found correlated with better response in univariate analysis, but it was not an independent factor. We believe the predictive effect of baseline neutrophils was dependent on its correlation with the administration of G-CSF. Because G-CSF was mostly given to patients with neutropenia in the study. Further studies are needed to find out the predictive effect on neutrophils, emphatically on TANs. Besides, KPS score >80, squamous carcinoma, negative N stage, M stage and high BED were found favorable indicators for PFS, which was consistent with previous studies (48, 49).

To better understand the underlying factors for the abscopal response, we compared the clinicopathological factors between the abscopal group and non-abscopal group. Radiation site, BED, fractionation regimens, radiation intention and synchronous chemotherapy were not associated with abscopal response in our results, but further studies are needed. The underlying mechanism of abscopal response has not been fully explored. The in-situ vaccination effect of radiation was widely accepted as the potential mechanism of abscopal response (50). Radiation released tumor associated antigens and could modulate immunological pathways, increasing tumor antigen presentation, activating tumor-specific cytotoxic T cells, as well as enhancing T-cell homing and function in tumors. Previous study reported that extracranial radiation showed more advantage in activating antitumor immune response (51). Also, enough BED of radiation is needed to induce the immunologic effect of radiation (52). Camphausen et al. found that the abscopal antitumor response was significantly more potent when mice were treated with greater BED. Tang et al evaluated multiple SBRT dose fractionation regimens (BED ranging from 96 to >100 for liver and lung metastases and found abscopal responses in 10% of patients (53). To be noted, these enhanced abscopal responses cannot be simply attributed to greater BED, since different fractionation regimens were used in those studies. Hypofractionation was reported more favorable in immunostimulants with a marked increase in T cell priming than conventional fractionation. Further studies are needed to figure out the best radiation regimen to maximize antitumor immunity.

The major limitation of our study was the retrospective study design, which failed to complete the elimination of bias in the selection of patients, even by using the PS matching method. The only way to eliminate all of the confounding factors and to verify the antitumor effects of G-CSF combined with radiation is to conduct a prospective randomized clinical trial (RCT). Our results provided strong evidence for clinical usage and to conduct the RCT subsequently. Despite the indicative effect of chemotherapy was not significant in the study, the contribution of systemic therapy to abscopal response reported herein cannot be defined, including chemotherapy, targeted therapy and immunotherapy.

In conclusion, our results showed that concurrent administration of G-CSF improved radiation response significantly and generated abscopal effects in NSCLC. These findings suggested that the combination of radiotherapy and G-CSF is potential a new strategy and should be further evaluated in prospective clinical trials.

Declarations

Ethics approval and consent to participate

The study has been reviewed and approved by the Ethics Committee of Shandong Cancer Hospital and Institute, China. This study has been conducted according to the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

F.F.T and J.M.Y. designed the study; D.Y.W. and P.L.W. collected data; F.F.T and T.W.Y. analyzed data and drafted the article. All of the authors qualified for authorship according to the requirements of the Journal, and all approved the final version for submission. The material is original research. All of the authors qualified for authorship according to the requirements of the Journal, and all approved the final version

for submission. The manuscript has not been previously published and has not been submitted for publication elsewhere while under consideration.

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Figures

Figure 1

The flowchart of patient selection

Figure 2

Progression-free survival according to the receipt of G-CSF before propensity-score matching (A) and after propensity-score matching (B).