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Original Paper

Induced Pluripotent Stem Cell Transplantation Improves Locomotor Recovery in Rat Models of Spinal Cord Injury: a Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Key Words
Spinal cord injury • Locomotor recovery • BBB score • Induced pluripotent stem cells • Rats • Meta-analysis

Abstract
Background/Aims: Spinal cord injury (SCI) has long been a subject of great interest in a wide range of scientific fields. Several attempts have been made to demonstrate motor function improvement in rats with SCI after transplantation of induced pluripotent stem cells (iPSC). This systematic review and meta-analysis was designed to summarize the effects of iPSC on locomotor recovery in rat models of SCI. ***Methods:*** We searched the publications in the PubMed, Medline, Science Citation Index, Cochrane Library, CNKI, and Wan-fang databases and the China Biology Medicine disc. Results were analyzed by Review Manager 5.3.0. The quality of evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology. ***Results:*** Six randomized controlled preclinical trials covering eight comparisons and including 212 rats were selected. The subgroup analyses were based on the following items: different SCI models, cell counts, iPSC sources, iPSC differentiations and transplantation methods. The pooled results indicated that iPSC transplantation significantly improved locomotor recovery of rats after SCI by sustaining beneficial effects, especially in the subgroups of contusion, moderate cell counts (5×10^5), source of human fetal lung fibroblasts, iPSC-neural precursors and intraspinal injection. ***Conclusion:*** Our meta-analysis of the effects of iPSC transplantation on locomotor function in SCI models is, to our knowledge, the first meta-analysis in this field. We conclude that iPSC transplantation improves locomotor recovery in rats with SCI, implicating this strategy as an effective therapy. However, more studies are required to validate our conclusions.

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Introduction

Spinal cord injury (SCI) is a devastating event, resulting in permanent neurological impairment and attendant social and economic losses [1, 2]. Due to the loss of sensory and motor capabilities, patients are usually rendered paraplegic or tetraplegic. Beyond that, bladder dysfunction, intestinal flora disturbance and cardiac problems represent the most lethal threat [3, 4]. Thus, improved strategies targeting these issues are urgently required.

To date, surgical interventions to decompress the spinal cord [5] and related rehabilitation [6] are the standard of care for acute SCI. However, no neuroprotective and regenerative therapies capable of producing directly beneficial effects are currently available [7]. It has been demonstrated that high-dose methylprednisolone may have good effects on SCI, but there remains no consensus on the efficacy of this approach [8].

Recent progress in stem cell research may be at the point of breaking this impasse [9]. A variety of stem cell types have shown their potential for transplantation, such as neural stem cells [10], mesenchymal stem cells [11], Schwann cells [12], embryonic stem cells [13] and more recently, induced pluripotent stem cells(iPSC) [14, 15]. Among these, iPSC has played a pivotal role in repairing the damaged spinal cord. Within the past 5 years, laboratories around the world have reported functional improvements following iPSC transplantation in animal models of SCI [16, 17]. This effect may be associated with significantly enhanced secretion of regenerative molecules and growth factors [7]. However, several studies have demonstrated poor survival of the cells and no significant functional recovery after the transplantation [18, 19].

Meta-analyses of controlled studies increase the power and precision of the estimated intervention effect and thus, represents a more powerful test of the null hypothesis than any of the individual studies alone [20]. To date, no quantitative data are available regarding locomotor recovery in rats following iPSC transplantation after SCI. As a result, we summarized and analyzed the history of basic research into iPSC transplantation in rats with SCI and evaluated the potential rat models as a platform for the development of iPSC therapy for SCI in the clinic.

Materials and Methods

Search strategy

Following the methodological recommendations of the Cochrane Collaboration and the PRISMA statement, the PubMed, Medline, Science Citation Index, Cochrane Library, CNKI, and Wan-fang electronic databases and the China Biology Medicine disc were searched to retrieve related studies. Notably, we searched the Medical Subject Heading (MeSH) terms “induced pluripotent stem cells”, “transplantation”, “spinal cord injury” and all related free words. The language, publication date, or publication status were not restricted.

Inclusion criteria

The inclusion criteria together with the PICO (Patient/ Participants, Intervention, Comparison and Outcome) approaches were established as follows:

1) Types of participants: laboratory rats of any breed, sex, body weight and age suffering contusion and compression of SCI were included.

2) Types of Intervention: we included the basic information of iPSC transplantation irrespective of cell sources, cell differentiation, transplantation method, cell count and time of transplantation. Labeling or transfection with markers for cellular tracing and imaging (such as green fluorescent protein) were included.

3) Types of comparison: the included publications contained at least two groups; iPSC transplantation and control groups. The control interventions comprised placebo (e.g. saline, culture medium or similar vehicle control). All rats underwent laminectomy followed by SCI before iPSC or control interventions.

4) Types of outcome evaluated: locomotor function was evaluated according to the open-field

(Basso, Beattie and Bresnahan, BBB) test. The 21-point BBB score was used to assess hind limb locomotion. The highest scores obtained using the BBB rating method represent normal function (coordinated gait, consistent toe clearance, predominant paw position is parallel throughout stance, consistent trunk stability, and tail is consistently up) [21]. It is a sensitive indicator of basic overground locomotion and can be used to evaluate limb movements and walking characteristics in an open-field environment [22].

5) Types of study design: randomized controlled animal trials were regarded as eligible for evaluation of iPSC transplantation in laboratory rats with SCI.

Exclusion criteria

Publications were excluded if they met one of the following criteria: no access to the full text; review; the mean and standard deviation (SD) of BBB scores were unavailable; BBB score is not in use; ischemic model; mouse model; chronic spinal cord injury model; use animal trials of low quality; concomitant injection with other cell types or use of adjuvant products (e.g. injectable hydrogel).

Definitions

We defined a ‘‘publication’’ as a discrete piece of work (including abstracts). Each publication may report data from more than one experiment. Each experiment may describe outcomes in several different experimental cohorts. The contrast between outcomes in a single intervention cohort with that in a control cohort we defined as a ‘‘comparison’’ [23].

Selection of studies

Based on the same selection criteria, two investigators (QC and GY) independently screened citations and publications identified by the initial search. Then, we selected potentially relevant titles, reviewed their abstracts and determined if the publications met the inclusion criteria. We also searched the reference lists in the selected publications identify any comparisons that were not identified in the original search. All disagreements were resolved by discussion until a consensus was reached between the two investigators. A third author was consulted if necessary.

Data extraction

The data were extracted independently by two reviewers (QC and GY) and were rechecked after the extraction by reading the titles, abstracts and the full text if necessary, according to the inclusion and exclusion criteria. We recorded the following information: first author’s name, publication year, the type of SCI, injury level of spinal cord, cells count, time of iPSC transplantation, cell sources and differentiation, iPSC transplantation method as well as rat breed, sex, body weight, age and number of rats per group. For each comparison, data were recorded for mean BBB score, SD and number of rats in each group. In publications with multiple comparisons, we considered only the data from the iPSC transplantation and control groups in each publication. We used GetData Graph Digitizer 2.25 to calculate the mean and SD of the BBB score for conditions for which data were only shown in graphs. Moreover, we planned to contact first or senior authors by email if necessary.

Study quality assessment

The quality of the included studies was assessed according to Cochrane Handbook for Systematic Reviews of Interventions version 5.3.0. Here, six items were widely used in previous studies [21, 24]: 1) random sequence generation; 2) allocation concealment; 3) blinding of outcome assessment; 4) incomplete outcome data; 5) selective reporting; 7) other bias. Every publication was assessed by two independent reviewers and each item was judged as “low risk”, “unclear” or “high risk”. Any discrepancy over bias assessment was resolved by group discussion.

Evidence quality assessment

Two authors (QC and GY) independently assessed the quality of evidence for the main outcomes and generated summary tables using the GRADE methodology (GRADEpro GDT, GRADEpro Guideline Development Tool, https://gradepro.org) [25]. The quality of evidence was judged as “high,” “moderate,” “low,” or “very low” for each outcome with six items: risk of bias, inconsistency, indirectness, imprecision, and publication bias. Any disagreement regarding evidence quality assessment was discussed and resolved.

Details of subgrouping

The subgroup analyses were based on the following items:

1) Different SCI models: compression (balloon-induced compression or clip compression) or contusion (set up by NYU Impactor or Infinite Horizon Impactor).

2) Different cell counts: different doses of cells for transplantation (cell counts $1×10^5$, $5×10^5$ and $1×10^6$).

3) Different iPSC sources: established from female (IMR90) human fetal lung fibroblasts or mouse embryonic fibroblasts.

4) Different iPSC differentiation: according to published protocols with slight modifications, iPSC were differentiated into neural precursors, oligodendrocyte progenitors or astrocytes under clonal conditions

5) Different transplantation methods: based on the different cell transplantation methods, subgroups of intrathecal (injected intrathecally between L3 and L4 or L4 and L5 through a 25 G needle for 30 s) or intraspinal (injected in the midline of the spinal cord at a depth of 1 mm below the dorsal surface) injections were established.

Statistical analysis

We used the Review Manager Software package (version 5.3.0; the Cochrane collaboration) to conduct the meta-analysis. For continuous outcomes, we reported pooled estimates as weighted mean differences (WMDs) with 95% CIs. WMDs were identified as statistically significant when $P < 0.05$. Statistical heterogeneity among studies and subgroups was evaluated with □^{2} and I^2 tests. Both fixed-effects and random-effects models were used to obtain summary WMDs. The fixed-effects model was employed in the absence of heterogeneity, otherwise the random-effects model was used. The subgroup analyses were adopted to analyze the source of heterogeneity. We analyzed the BBB scores according to the time observed (1–7 weeks) after SCI.

Results

Selection of publications

A total of 79 publications were initially identified after computer and manual literature searches. After selecting potentially relevant titles, and reviewing abstracts and full texts if necessary, a total of six publications covering eight comparisons published from 2011 to 2017 were included in the meta-analysis. The detailed flow diagram of the publication selection process is shown in Fig. 1.

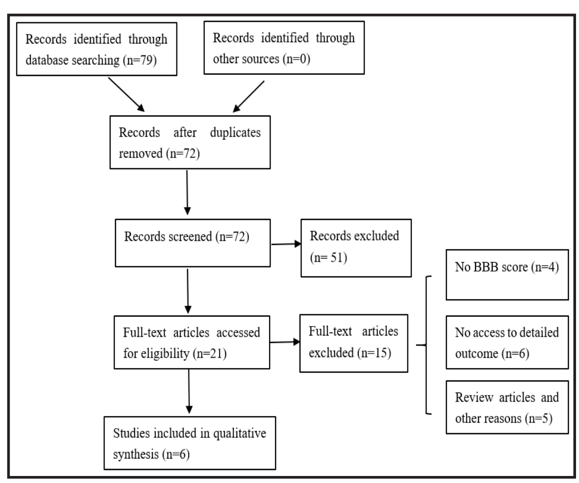

Fig. 1. Flow chart illustrating the literature search strategy and the different phases of publication eligibility assessment. Including publications: Jiri Ruzicka et al. 2017 [26]; Nataliya Romanyuk et al. 2015 [27]; Takashi Amemori et al. 2015 [28]; Angelo H. All et al. 2015 [29]; Jin Young Hong et al. 2014 [30]; Koichi hayashi et al. 2011[31].

Description of comparisons

Characteristics of the included comparisons are detailed in Table 1 and Table 2. Overall, 212 experimental rats were included. In terms of ways to induce SCI, the contusion models were adopted for four comparisons and the compression model was used for the other four comparisons. For the cell counts, rats in iPSC groups received injections of $5×10^5$ iPSC in five comparisons, $1×10^5$ iPSC in two comparisons and $1×10^6$ iPSC in one comparison. For cells sources, most of the comparisons used iPSC established from female human fetal lung fibroblasts, except three comparisons, in which mouse embryonic fibroblasts were used

Table 1. Description of included publications. SCI: spinal cord injury; FHFLF: female (IMR90) human fetal lung fibroblasts; MEF: mouse embryonic fibroblasts

Author and Year	Settings	SCI model	Injury level	Cell count	Transplantation time	Cell sources	Cell differentiation	Transplantation methods
Jiri Ruzicka 2017 [26]	Czech Republic	Compression	T8	5×10^5	7d	FHFLF	Neural precursors	Intraspinal injection
Nataliya Romanyuk 2015 [27]	Czech Republic	Compression	T8-T9	5×10^5	7d	FHFLF	Neural precursors	Intraspinal injection
Takashi Amemori 2015 [28]	Czech Republic	Compression	T8	5×10^5	7d	FHFLF	Neural precursors	Intrathecal intraspinal injection
Angelo H. All 2015 [29]	America	Contusion	T8	5×10^5	24h	FHFLF	Oligodendrocyte progenitors	Intraspinal injection
Jin Young Hong 2014 [30]	Korea	Contusion	T9	1×10^6	9d	MEF	Neural precursors	Intraspinal injection
Koichi hayashi 2011 [31]	Japan	Contusion	T9–T10	1×10^5	3d 7d	MEF	Astrocytes	Intraspinal injection

Table 2. Characteristics of included experimental rats. iPSC: Induced pluripotent stem cells

Author and Year	Breed and gender	Body weight	Age	Rats of iPSC group	Rats of control group
Jiri Ruzicka 2017 [26]	Male Wistar rats	285-315g	10-week-old	24	16
Nataliya Romanyuk 2015 [27]	Male Wistar rats	270-300g	10-week-old	21	22
Takashi Amemori 2015 [28]	Male Wistar rats	270-300g	10-week-old	18	20
Angelo H. All 2015 [29]	Female Lewis rats	200-220g	10-week-old	12	12
Jin Young Hong 2014 [30]	Female Sprague-Dawley rats	230–250 g	12-week-old	12	9
Koichi Hayashi 2011 [31]	Female Sprague-Dawley rats	not mentioned	8-week-old	29	17

as iPSC sources. For cell differentiation, with slight modifications, iPSC were differentiated into neural precursors in five comparisons, oligodendrocyte progenitors in one comparison and astrocytes in two comparisons under clonal conditions. For iPSC transplantation methods, most comparisons transplanted iPSC by intraspinal injection, while intrathecal injection was used for only 1 comparison (Table 1).

As shown in Table 2, we next characterized the basic information of the experimental rats included in the following terms: breed, sex, body weight, and age.

Methodological study quality assessment

A summary of the methodological domain assessment for each comparison is shown in Fig. 2. Only three comparisons did not clearly mention the blinding of outcome assessment and other bias remained unclear in six comparisons. Overall, the risk of bias was considered to be low.

To facilitate understanding, we made Table 3-7 to present the data (WMDs and heterogeneity) of all the subgroups (iPSC vs control group) straightforward.

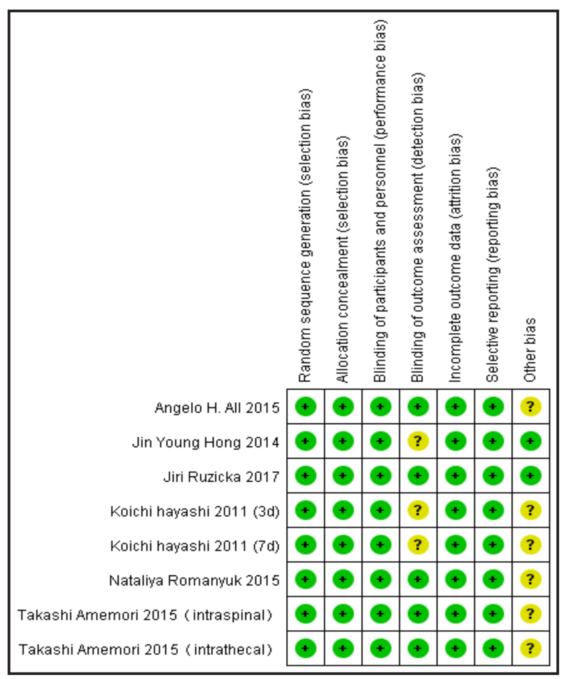

Fig. 2. Risk of bias summary: review of authors' judgments about each risk of bias item for each included comparison. Including publications: Jiri Ruzicka et al. 2017 [26]; Nataliya Romanyuk et al. 2015 [27]; Takashi Amemori et al. 2015 [28]; Angelo H. All et al. 2015 [29]; Jin Young Hong et al. 2014 [30]; Koichi hayashi et al. 2011[31].

BBB scores in subgroups of different types of SCI models

BBB scores at 1–7 weeks after transplantation. As shown in Figures 3A–3G, no significant difference was found between the iPSC and control groups in terms of BBB score of the contusion subgroups at 1-7 weeks after iPSC transplantation. The BBB scores of the compression subgroups were significantly higher in the iPSC groups than those in the control groups at 1-7 weeks after iPSC transplantation (WMD = 3.77; 95% CI: 3.17–4.36; $P < 0.001$; WMD = 4.33; 95% CI: 4.02–4.64; $P < 0.001$; WMD = 4.05; 95% CI: 3.28–4.82; $P < 0.001$; WMD = 3.86; 95% CI: 3.09–4.63; $P < 0.001$; WMD = 4.14; 95% CI: 3.34–4.93; $P < 0.001$; WMD = 4.12; 95% CI: 3.38–4.86; $P < 0.001$; WMD = 4.58; 95% CI: 3.69–5.48; $P < 0.001$). The

Table 3. Results of weighted mean differences (WMDs) and heterogeneity in subgroups of different types of SCI models (iPSC vs control group) * means P>0.05; Subgroup means the heterogeneity between subgroups

Observing time	WMDs			Heterogeneity(I^2)			
	Compression	Contusion	Total	Compression	Contusion	Total	Subgroup
1week	3.77	-0.36*	1.77	84%	98%	98%	88.9%
2weeks	4.33	1.01*	2.75	61%	92%	97%	95.5%
3weeks	4.05	0.70*	2.41	96%	90%	98%	95.1%
4weeks	3.86	0.99*	2.48	95%	96%	98%	91.1%
5weeks	4.14	1.11*	2.65	96%	96%	98%	90.0%
6weeks	4.12	0.88*	2.49	95%	89%	98%	95.8%
7weeks	4.58	1.07*	2.88	97%	97%	98%	87.6%

Table 4. Results of weighted mean differences (WMDs) and heterogeneity in subgroups of different doses of cells (iPSC vs control group) * means P>0.05; NA means not applicable; Subgroup means the heterogeneity between subgroups

Observing time	WMDs				Heterogeneity(I^2)				
	5×10^5	1×10^5	1×10^6	Total	5×10^5	1×10^5	1×10^6	Total	Subgroup
1week	2.57	-0.62*	2.25	1.77	99%	96%	NA	98%	0%
2weeks	3.99	-0.04*	1.68	2.75	92%	0%	NA	97%	97.5%
3weeks	3.47	-0.30*	2.35	2.41	97%	50%	NA	98%	92.4%
4weeks	3.60	-0.43*	2.01	2.48	98%	57%	NA	98%	92.2%
5weeks	3.98	-0.30*	1.90	2.65	95%	0%	NA	98%	97.5%
6weeks	3.68	-0.41*	1.90	2.49	96%	81%	NA	98%	89.5%
7weeks	4.39	-0.62*	2.30	2.88	96%	0%	NA	98%	97.9%

Table 5. Results of weighted mean differences (WMDs) and heterogeneity in subgroups of different iPSC sources (iPSC vs control group) * means P>0.05; Subgroup means the heterogeneity between subgroups; FHFLF means female (IMR90) human fetal lung fibroblasts; MEF means mouse embryonic fibroblasts

Observing time	WMDs			Heterogeneity(I^2)			
	FHFLF	MEF	Total	FHFLF	MEF	Total	Subgroup
1week	2.57	0.43*	1.77	99%	94%	98%	47.7%
2weeks	3.99	0.48*	2.75	92%	85%	97%	95.4%
3weeks	3.47	0.52*	2.41	97%	93%	98%	86.7%
4weeks	3.60	0.36*	2.48	98%	93%	98%	90.4%
5weeks	3.98	0.33*	2.65	95%	94%	98%	92.9%
6weeks	3.68	0.45*	2.49	96%	92%	98%	92.3%
7weeks	4.39	0.21*	2.88	96%	94%	98%	91.8%

Table 6. Results of weighted mean differences (WMDs) and heterogeneity in subgroups of different iPSC differentiation (iPSC vs control group) * means P>0.05; NA means not applicable; Subgroup means the heterogeneity between subgroups

Observing time	WMDs				Heterogeneity(I^2)				
	Neural precursors	Oligodendrocyte progenitors	Astrocytes	Total	Neural precursors	Oligodendrocyte progenitors	Astrocytes	Total	Subgroup
1week	3.51	-2.47	-0.62*	1.77	86%	NA	96%	98%	98.9%
2weeks	3.86	2.50	-0.04*	2.75	93%	NA	0%	97%	97.2%
3weeks	3.73	1.04	-0.30*	2.41	96%	NA	50%	98%	95.3%
4weeks	3.49	2.68	-0.43*	2.48	97%	NA	57%	98%	92.3%
5weeks	3.67	3.36	-0.30*	2.65	98%	NA	0%	98%	97.7%
6weeks	3.66	1.86	-0.41*	2.49	97%	NA	81%	98%	88.0%
7weeks	4.15	3.59	-0.62	2.88	97%	NA	0%	98%	98.4%

Table 7. Results of weighted mean differences (WMDs) and heterogeneity in subgroups of different transplantation methods (iPSC vs control group) * means P>0.05; NA means not applicable; Subgroup means the heterogeneity between subgroups

Observing time	WMDs			Heterogeneity(I^2)			
	Intraspinal	Intrathecal	Total	Intraspinal	Intrathecal	Total	Subgroup
1week	1.36*	4.67	1.77	99%	NA	98%	91.4%
2weeks	2.56	4.03	2.75	98%	NA	97%	78.5%
3weeks	2.43	2.26	2.41	98%	NA	98%	0%
4weeks	2.52	2.17	2.48	98%	NA	98%	0%
5weeks	2.69	2.37	2.65	98%	NA	98%	0%
6weeks	2.48	2.50	2.49	98%	NA	98%	0%
7weeks	2.91	2.71	2.88	99%	NA	98%	0%

corresponding heterogeneities were moderate (I^2 = 61%) at 2 weeks after transplantation but were high at 1, and 3–7 weeks after transplantation (I^2 = 84%, 96%, 95%, 96%, 95% and 97%, respectively).

Notably, the overall BBB scores were significantly higher in the iPSC groups than those in the control groups at 1-7 weeks (WMD = 1.77; 95% CI: 0.20–3.35; P = 0.03;WMD = 2.75; 95% CI, 1.78–3.72; P < 0.001; WMD = 2.41; 95% CI, 1.35–3.47; P < 0.001; WMD = 2.48; 95% CI, 1.55–3.41; P < 0.001; WMD = 2.65; 95% CI, 1.55–3.75; P < 0.001; WMD = 2.49; 95% CI, 1.39–3.59; P < 0.001 and WMD = 2.88; 95% CI, 1.64–4.12; P < 0.001, respectively) after iPSC transplantation. The total heterogeneities were high at 1-7 weeks (I^2 = 98%, 97%, 98%, 98%, 98%, 98% and 98%, respectively) after transplantation. The heterogeneities between subgroups were also high (I^2 = 88.9%, 95.5%, 95.1%, 91.1%, 90.0%, 95.8% and 87.6%, respectively). All the results favored the iPSC group, which suggested a protective effect.

BBB scores in subgroups of different doses of cells

BBB score at 1–6 weeks after transplantation. Comparisons were divided into three subgroups, which received iPSC by injection at cell counts of $5×10^5$, $1×10^5$ and $1×10^6$, respectively. As indicated in Fig. 4A–4F, the iPSC and control groups exhibited similar changes in BBB scores at 1–6 weeks after iPSC transplantation. Specifically, the BBB scores in the $5×10^5$ subgroup were significantly higher in the iPSC groups than those in the control groups (WMD = 2.57; 95% CI: 0.50–4.63; P = 0.01; WMD = 3.99; 95% CI: 3.37–4.61; P < 0.001; WMD = 3.47; 95% CI: 2.53–4.41; P < 0.001; WMD = 3.60; 95% CI: 2.70–4.50; P < 0.001; WMD = 3.98; 95% CI: 3.25–4.71; P < 0.001 and WMD = 3.68; 95% CI: 2.80–4.55; P < 0.001, respectively). The corresponding heterogeneities were high (I^2 = 99%, 92%, 97%, 98%, 95% and 96%, respectively). The BBB scores in the $1×10^6$ subgroup were significantly higher in the iPSC groups than those in the control groups (WMD = 2.25; 95% CI, 1.45–3.05; P < 0.001; WMD = 1.68; 95% CI, 0.98-2.38; P < 0.001; WMD = 2.35; 95% CI, 1.65–3.05; P < 0.001; WMD = 2.01; 95% CI, 1.41–2.61; P < 0.001; WMD = 1.90; 95% CI, 1.42–2.38; P < 0.001 and WMD = 1.90; 95% CI, 1.39–2.41; P < 0.001, respectively). However, there were no significant differences in the BBB scores between the iPSC and control groups in the $1×10^5$ subgroups. Notably, because we analyzed the same included comparisons as those in the different SCI model subgroups, we achieved the same results in terms of the overall BBB score and total heterogeneities, favoring the iPSC groups. The heterogeneity between subgroups was also high at 2–6 weeks (I^2 = 97.5%, 92.4%, 92.2%, 97.5% and 89.5%, respectively) after transplantation, except at 1 week after transplantation (I^2 = 0%).

BBB score at 7 weeks after transplantation. As indicated in Fig. 4G, the BBB scores in the $5×10^5$ subgroup were significantly higher in the iPSC group than those in the control groups at 7 weeks after transplantation (WMD = 4.39; 95% CI: 3.55–5.23; P < 0.001). The corresponding heterogeneity was high (I^2 = 96%). Conversely, the BBB scores in the $1×10^5$ subgroup were significantly lower in the iPSC groups than those in the control groups at 7 weeks (WMD = -0.62; 95% CI: -1.23 – -0.02; P = 0.04) after transplantation. The relevant heterogeneity was zero (I^2 = 96%). Notably, the overall BBB scores were significantly higher in the iPSC groups than those in the control groups (WMD = 2.88; 95% CI: 1.64–4.12; P < 0.001). The total heterogeneity and the heterogeneity between subgroups was high (I^2 = 98% and 97.9%, respectively).

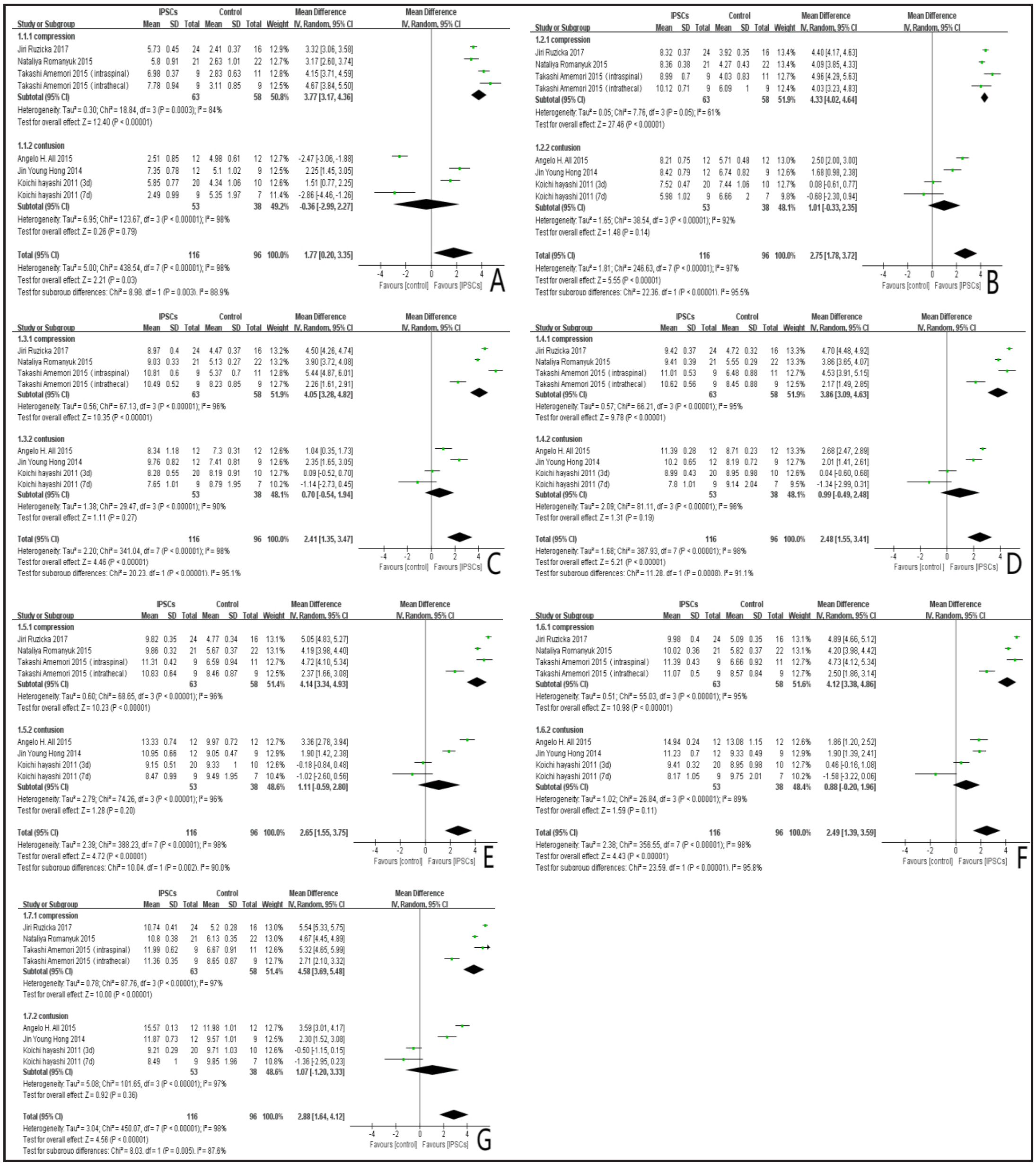

Fig. 3. Forest plot of the differences in the BBB scores of the iPSC and control groups in different injury model subgroups at different time-points after transplantation. (A–G) At 1–7 weeks, respectively, after iPSC transplantation. Including publications: Jiri Ruzicka et al. 2017 [26]; Nataliya Romanyuk et al. 2015 [27]; Takashi Amemori et al. 2015 [28]; Angelo H. All et al. 2015 [29]; Jin Young Hong et al. 2014 [30]; Koichi hayashi et al. 2011[31].

BBB score in subgroups of different iPSC sources

BBB scores at 1–7 weeks after transplantation. Based on the different iPSC sources, we divided the comparisons into two subgroups of iPSC established from female (IMR90) human fetal lung fibroblasts or from mouse embryonic fibroblasts. As shown in Figures 5A–5G, the BBB scores of the female (IMR90) human fetal lung fibroblast subgroup were significantly higher in the iPSC groups than those in the control groups and the corresponding heterogeneities were high (data not shown because the included comparisons are the same those in the contusion subgroups). However, there was no significant difference in the BBB scores of the mouse embryonic fibroblast subgroups between the iPSC and control groups.

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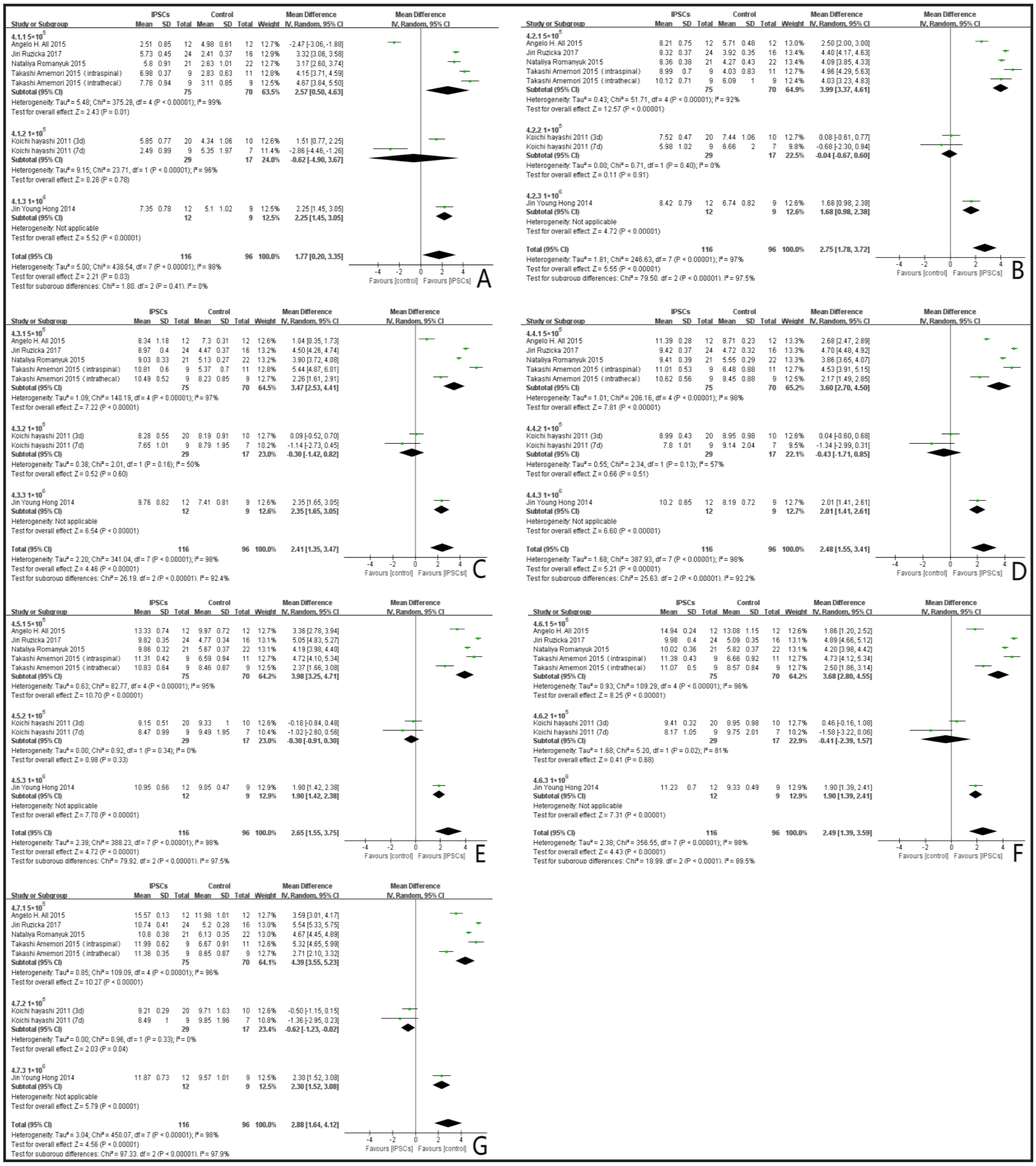

Fig. 4. Forest plot of the differences in the BBB scores of the iPSC and control groups in different cell counts subgroups at different time-points after transplantation. (A–G) At 1–7 weeks, respectively, after iPSC transplantation. Including publications: Jiri Ruzicka et al. 2017 [26]; Nataliya Romanyuk et al. 2015 [27]; Takashi Amemori et al. 2015 [28]; Angelo H. All et al. 2015 [29]; Jin Young Hong et al. 2014 [30]; Koichi hayashi et al. 2011[31].

Notably, because we analyzed the same included comparisons as those in the different SCI model subgroups, we obtained the same results in terms of the overall BBB score and total heterogeneities, favoring the iPSC groups, which suggested a protective effect. There were significant differences in the heterogeneities between the subgroups (I^2 = 47.7%, 95.4%, 86.7%, 90.4% and 89.5%, respectively).

BBB score in subgroups of different iPSC differentiation

BBB scores at 1–7 weeks after transplantation. According to published protocols with slight modifications, iPSC were differentiated into neural precursors, oligodendrocyte progenitors or astrocytes under clonal conditions, representing three subgroups. As

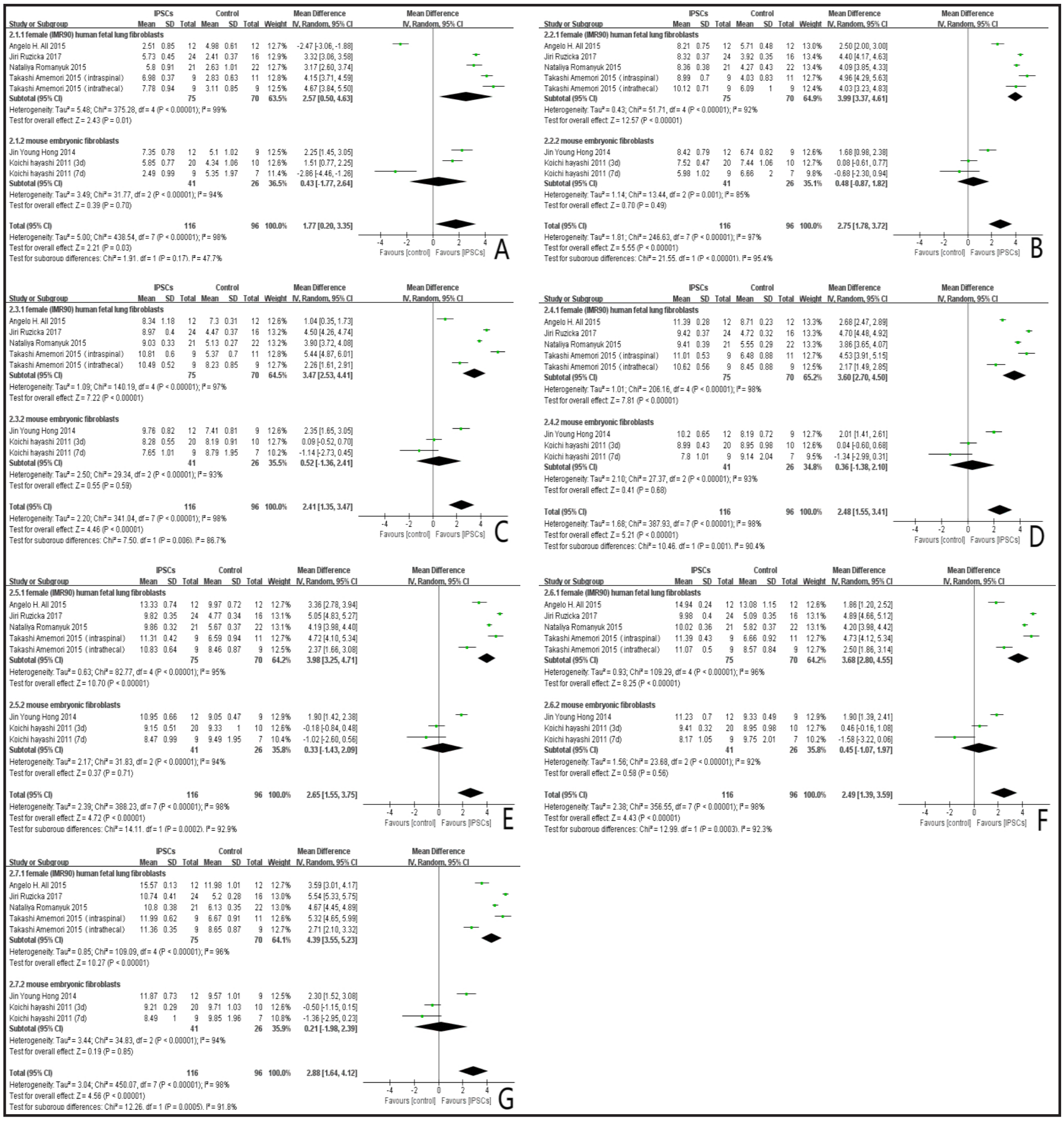

Fig. 5. Forest plot of the differences in the BBB scores of the iPSC and control groups in different iPSC source subgroups at different time-points after transplantation. (A–G) At 1–7 weeks, respectively, after iPSC transplantation. Including publications: Jiri Ruzicka et al. 2017 [26]; Nataliya Romanyuk et al. 2015 [27]; Takashi Amemori et al. 2015 [28]; Angelo H. All et al. 2015 [29]; Jin Young Hong et al. 2014 [30]; Koichi hayashi et al. 2011[31].

shown in Figures 6A–6G, we observed similar changes in the BBB scores between the iPSC and control groups. Specifically, the BBB scores of the neural precursor subgroup were significantly higher in the iPSC groups than those in the control groups (WMD = 3.51; 95% CI: 2.90–4.13; $P < 0.001$; WMD = 3.86; 95% CI: 3.17–4.56; $P < 0.001$; WMD = 3.73; 95% CI: 2.97–4.50; $P < 0.001$; WMD = 3.49; 95% CI: 2.64–4.34; $P < 0.001$; WMD = 3.67; 95% CI: 2.65–4.68; $P < 0.001$; WMD = 3.66; 95% CI: 2.70–4.62; $P < 0.001$ and WMD = 4.15; 95% CI: 3.20–5.10; $P < 0.001$, respectively). The relevant heterogeneities were high (I^2 = 86%, 93%, 96%, 97%, 98%, 97% and 97%, respectively). The BBB scores in the oligodendrocyte progenitor subgroup were lower in the iPSC groups than those in the control groups at 1 week after iPSC transplantation, but were significantly higher at 2–7 weeks. Given that the same comparisons were included in the astrocytes subgroup, we achieved the same results as those obtained in the $1{\times}10^5$ subgroup at 1–7 weeks after transplantation. Furthermore, the overall BBB scores

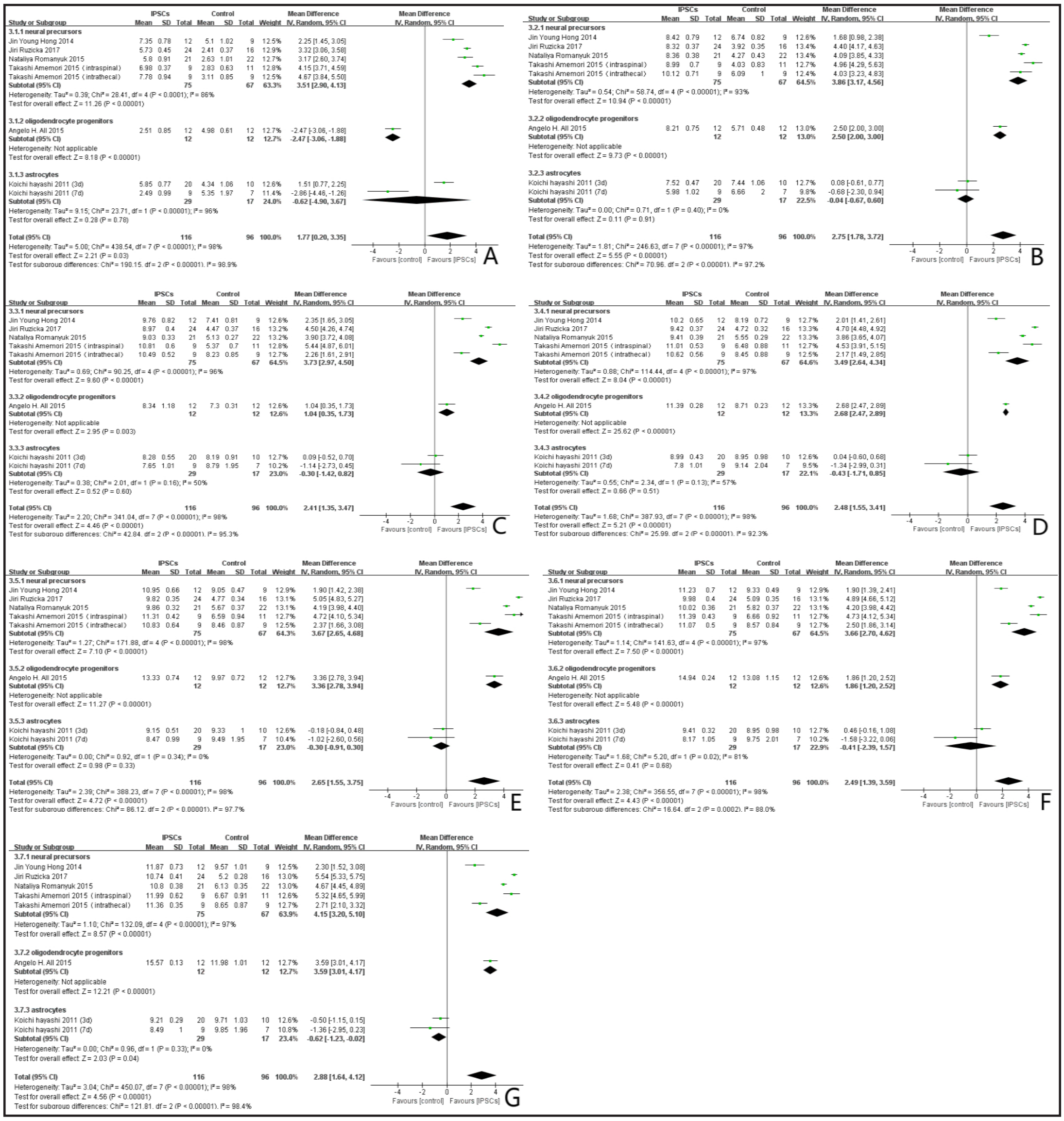

Fig. 6. Forest plot of the differences in the BBB scores of the iPSC and control groups in different iPSC differentiation subgroups at different time-points after transplantation. (A–G) At 1–7 weeks, respectively, after iPSC transplantation. Including publications: Jiri Ruzicka et al. 2017 [26]; Nataliya Romanyuk et al. 2015 [27]; Takashi Amemori et al. 2015 [28]; Angelo H. All et al. 2015 [29]; Jin Young Hong et al. 2014 [30]; Koichi hayashi et al. 2011[31].

and total heterogeneities were the same as those in the other subgroups, favoring the iPSC groups, which suggested a protective effect. The heterogeneities between subgroups were also different (I^2 = 98.9%, 97.2%, 95.3%, 92.3%, 97.7%, 88.0% and 98.4%, respectively).

BBB scores in subgroups of different transplantation methods

BBB scores at 1 week after transplantation. We divided the subgroups according to the different transplantation methods (intrathecal or intraspinal injection). As shown in Fig. 7A, there was no significant difference between the iPSC and control groups in terms of BBB scores in the intraspinal injection subgroup. In contrast, the BBB scores in the intrathecal injection subgroup were significantly higher in the iPSC groups than those in the control groups (same data as the other subgroups). Notably, because we analyzed the same included comparisons as those in the subgroups of different SCI models, we obtained the same results

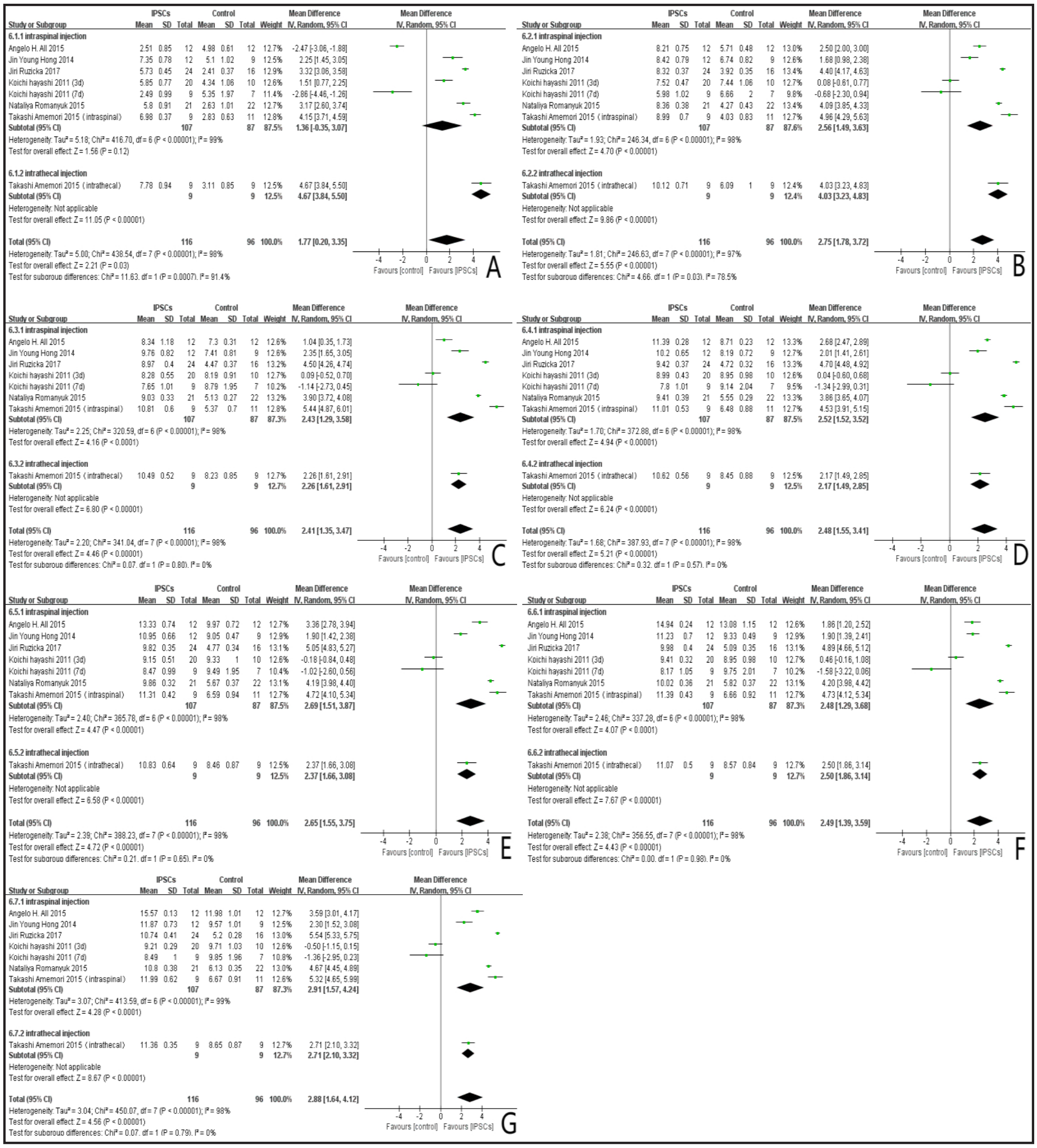

Fig. 7. Forest plot of the differences in the BBB scores of the iPSC and control groups in different transplantation method subgroups at different time-points after transplantation. (A–G) At 1–7 weeks, respectively, after iPSC transplantation. Including publications: Jiri Ruzicka et al. 2017 [26]; Nataliya Romanyuk et al. 2015 [27]; Takashi Amemori et al. 2015 [28]; Angelo H. All et al. 2015 [29]; Jin Young Hong et al. 2014 [30]; Koichi hayashi et al. 2011[31].

in terms of the overall BBB scores and total heterogeneities, favoring the iPSC groups, which suggested a protective effect. The heterogeneity between subgroups was high (I^2 = 91.4%).

BBB scores at 2–7 weeks after transplantation. As shown in Figures 7B–7G, similar changes in BBB scores between the iPSC and control groups were found at 2–7 weeks after iPSC transplantation. Specifically, the BBB scores of the intraspinal injection subgroup were significantly higher in the iPSC groups than those in the control groups (WMD = 2.56; 95% CI: 1.49–3.63; P < 0.001; WMD = 2.43; 95% CI: 1.29–3.58; P < 0.001; WMD = 2.52; 95% CI: 1.52–3.52; P < 0.001; WMD = 2.69; 95% CI: 1.51–3.87; P < 0.001; WMD = 2.48; 95% CI: 1.29–3.68; P < 0.001 and WMD = 2.91; 95% CI:1.57–4.24; P < 0.001, respectively). The relevant heterogeneities were high (I^2 = 98%, 98%, 98%, 98%, 98% and 99%, respectively). The BBB scores in the intrathecal injection subgroup were significantly higher in the iPSC groups than those in the control groups (same data as the other subgroups). Notably, because we analyzed the same included comparisons, we obtained the same data in the overall BBB scores and total heterogeneities, favoring the iPSC groups. The heterogeneities between subgroups were also different (I^2 = 78.5%, 0%, 0%, 0%, 0% and 0%, respectively).

Level of evidence assessment

The GRADE evidence profiles are shown in Table 8. The GRADE level of evidence was moderate for locomotor recovery in rats with SCI at 1–7 weeks after iPSC transplantation.

Reasons to reject iPSC-SCI related publications

We rejected 3 iPSC-SCI related publications according to our filter criterion. As a result, Table 9 was made to list these controversial publications with clear explanation to increase the rigor of our study.

Discussion

In this meta-analysis, we comprehensively reviewed the current literature and demonstrated that iPSC promote locomotor recovery in rats with SCI. We also provide a description of the different factors underlying SCI recovery, including SCI models, doses of cells, iPSC sources, iPSC differentiation and transplantation methods, which are considered to play critical roles in the repair process. In reviewing the literature, no pre-clinical evidence was summarized on iPSC transplantation in SCI models. Given this, the meta-analysis of iPSC on locomotor recovery is, to our knowledge, the first meta-analysis in this field.

Table 8. GRADE evidence profile. Moderate quality = further research is likely to have an important impact on our confidence in the estimate of the effect and may change the estimate

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Induced pluripotent stem cells	Control	Relative (95% CI)	Absolute		
Locomotor recovery at 1 week after transplantation (follow-up mean 1 weeks; measured with: BBB score; Better indicated by lower values)												
8	Randomised trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	116	96	-	MD 1.77 higher (0.2 to 3.35 higher)	⊕⊕⊕O MODERATE	CRITICAL
Locomotor recovery at 2 weeks after transplantation (follow-up mean 2 weeks; measured with: BBB score; Better indicated by lower values)												
8	Randomised trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	116	96	-	MD 2.72 higher (1.41 to 4.03 higher)	⊕⊕⊕O MODERATE	CRITICAL
Locomotor recovery at 3 weeks after transplantation (follow-up mean 3 weeks; measured with: BBB score; Better indicated by lower values)												
8	Randomised trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	116	96	-	MD 2.41 higher (1.35 to 3.47 higher)	⊕⊕⊕O MODERATE	CRITICAL
Locomotor recovery at 4 weeks after transplantation (follow-up mean 4 weeks; measured with: BBB score; Better indicated by lower values)												
8	Randomised trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	116	96	-	MD 2.48 higher (1.55 to 3.41 higher)	⊕⊕⊕O MODERATE	CRITICAL
Locomotor recovery at 5 weeks after transplantation (follow-up mean 5 weeks; measured with: BBB score; Better indicated by lower values)												
8	Randomised trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	116	96	-	MD 2.65 higher (1.55 to 3.75 higher)	⊕⊕⊕O MODERATE	CRITICAL
Locomotor recovery at 6 weeks after transplantation (follow-up mean 6 weeks; measured with: BBB score; Better indicated by lower values)												
8	Randomised trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	116	96	-	MD 2.49 higher (1.39 to 3.59 higher)	⊕⊕⊕O MODERATE	CRITICAL
Locomotor recovery at 7 weeks after transplantation (follow-up mean 7 weeks; measured with: BBB score; Better indicated by lower values)												
8	Randomised trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	116	96	-	MD 2.88 higher (1.64 to 4.12 higher)	⊕⊕⊕O MODERATE	CRITICAL

Table 9. Reasons to reject iPSC-SCI related publications. SCI: spinal cord injury; FHFLF: female (IMR90) human fetal lung fibroblasts; AHDF: Adult human dermal fibroblasts

Author and Year	Animal model	SCI model and transplanting time	Immuno-suppression	Cell sources	Reasons for exclusion
Samuel E. Nutt; 2013 [43]	Adult female Long–Evans rats	Contusion; 4 weeks after injury	No	FHFLF	1) An early chronic injury model was established in this study which is different from acute and sub-acute phases in microenvironment; 2) BBB score was not used.
Yuriy Pomeshchik; 2015 [44]	Adult female C57BL/6J mice	Contusion; 7 days after SCI	Yes; Via injections of tacrolimus	AHDF	1) They used mouse models which is different from rat models; 2) BMS score instead of BBB score was used.
Clara López-Serrano; 2016 [45]	Adult female SD rats	Contusion; 0 and 7days postinjury	Yes; Via injections of FK506	AHDF	1) The derivation protocol of neural lineage cells from iPSC was different; 2) Adult cells were used which are more vulnerable to changes induced by the injured environment than fetal cells.

To arrive at robust conclusions, data were discussed when an outcome was reported from at least three simultaneous comparisons. In our present meta-analysis, we included six publications with eight comparisons [26-31]. The most obvious finding to emerge from our analyses was that in rats with SCI, iPSC transplantation significantly promotes the locomotor recovery according to the BBB score. A possible explanation for this might be that iPSC are one of the most widely used cell types for transplantation performed to recover the functions impaired as a result of injury [32]. In addition, the mechanism by which iPSC transplantation mediated functional improvements after SCI is multifaceted [33-35] although it is commonly accepted that iPSC transplantation can [7]: 1) reduce the area of syringomyelia and increase the area of spared tissue; 2) promote local microvascular regeneration and nerve regeneration for the repair of damaged cells; 3) reduce inflammation and inhibit oxidative stress after SCI; and 4) improve axonal growth and reconstruction of neural pathways by secreting substrates. Moreover, our findings are consistent with the data obtained in the study reported by Führmann et al., which demonstrated that transplantation of pluripotent stem cells and their differentiated progeny has the potential to regenerate functional pathways and improve locomotor function after SCI [36].

It is worth noting that several intriguing discoveries were made because of the subgroup analyses. First, in the compression subgroups, the BBB score was significantly higher in the iPSC groups than that in the control groups at 1–7 weeks after transplantation. This suggests that iPSC transplantation exerts a significantly favorable influence on locomotor recovery in a rat model of SCI, especially in the compression injury models, rather than in the contusion models. This result may be explained by the fact that all the studies of compression models used the same parameters with a balloon-induced injury lesion. However, different impactors and parameters were adopted in the studies of contusion models. Thus, more studies with compression injury models should be conducted to verify the beneficial effects of iPSC on contusion injury models. Namely, in compression models, the inflammation and edema, even hemorrhage, are increasing to a similar level after SCI, which can significantly change the intramedullary pressure to a certain extent. Accordingly, the pathophysiology after the primary injury is likely to result in an undesirable microenvironment for iPSC transplantation, with swelling or the spinal cord as well as intramedullary hemorrhagic necrosis hindering tissue repair in compression models. Second, our subgroup analyses of different cell counts indicated that iPSC at doses of $5{\times}10^5$ improve the locomotor recovery of rats with SCI at 1–7 weeks after transplantation. This indicates that the optimal dose for iPSC transplantation is $5{\times}10^5$, which is consistent with the results of earlier studies [33, 37], although further relevant studies should be conducted to validate these results. We also found that after transplantation, rats showed better functional recovery in subgroups transplanted with iPSC induced from female (IMR90) human fetal lung fibroblasts, which may survive at the lesion site. This finding is in accordance with previous findings [38] showing that transplantation of iPSC derived from this source also facilitate axonal regrowth as well as improved functional and electrophysiological recovery.

Next, we found that after grafting of human iPSC-derived neural precursors into the injured spinal cord of rats, the locomotor recovery was significantly promoted in the neural precursor groups compared with that observed in the control groups. It can be speculated that this is because the transplanted cells differentiate mainly into neurons and form synapses, improve axonal reconstruction and angiogenesis, and prevent demyelination [39]. Last, but not least, our analyses indicate that intraspinal implantation is an appropriate transplantation method, which was adopted in most of the included studies. These results are in accordance with recent publications [28, 40] indicating that direct injection of human iPSC promotes locomotor recovery. However, this type of transplantation may cause additional damage leading to further damage to the injured spinal cord [41]. Notably, one publication included in our meta-analysis [28] demonstrated that intrathecal injection had a moderate therapeutic benefit on SCI via a paracrine mechanism that does not require the cells to be present in the tissue. However, the relevant publications are too few to confirm the efficacy of this method.

Surprisingly, relatively high heterogeneity was found in many subgroup analyses. There are, however, several possible explanations. As shown in Table 2, a total of 212 experimental rats were included in our meta-analysis, which consisted of different breeds, sexes, body weights and age. In addition, compared with patients, rodent models are more vulnerable to unpredictable factors, such as different parameters of injury impactors (NYU or Infinite Horizon Impactor, height of impactors and injury level, etc.), operative details (surgical procedures, time, blood loss, etc.) and post-operative nursing (temperature, humidity and adjuvant therapy, etc.). As a result, these inconsistencies may lead to the heterogeneity under this circumstance. It can also be assumed that fewer publications in subgroups may have some unexpected impacts on the results and explanations. Thus, a higher heterogeneity might be achieved which requires much more publications to be conducted to verify our findings. Yet despite this, all the publications included in our analyses evaluated locomotor function in rats with SCI using BBB scores, which is a sensitive and reliable method used to assess the behavioral changes of rats [42]. In addition, in this review, the BBB score in most iPSC groups were at least 2 points higher than those in the corresponding control groups, suggesting that iPSC transplantation promotes locomotor function in rats subjected to SCI.

We focused on the studies with acute and sub-acute injury models and exclude those with chronic injury models. They have different factors in terms of microenvironment which may have a significant influence in the functional recovery. As a result, we analyzed the publications of acute and sub-acute phases of spinal cord injury to make our conclusions clear and definite.

We also eliminate one publication with a negative outcome for iPSC-derived cells according to our filter criteria [43-45]. First, they used Adult human dermal fibroblasts as cells source. Regarding the source of NSCs, the derivation protocol of neural lineage cells from iPSC may be crucial to obtain different NSCs. Second, adult cells are more vulnerable to changes induced by the injured environment than fetal cells which is the main reason for a negative outcome. Furthermore, the use of certain factors during reprogramming may enhance neurite outgrowth, maturation, and expression of different neural markers, influencing engraftment and differentiation within the injured nervous system. Thus, we will keep a watchful eye on the field of adult human dermal fibroblasts and more studies about the functional effect of these cells on SCI should be conducted in the future.

The effect of immunosuppression on graft survival should not be neglected. In our meta-analysis, only one publication [27] mentioned the use of triple drug immunosuppression containing Cyclosporine A (10 mg/kg), azathioprine sodium (2 mg/kg), methylprednisolone (2 mg/kg, tapered to 0.5 mg/kg) and methylprednisolone (2 mg/kg, tapered to 0.5 mg/kg) to prevent graft rejection. Generally, it is necessary to use initial combined immunosuppressive therapy in order to achieve consistent cell survival at intervals of 2–2.5 months after grafting [46]. Few publications are included, as a result, we need more studies and evidence to valid this method.

Notably, GRADE provides explicit criteria for rating the quality of evidence that include study design, risk of bias, imprecision, inconsistency, indirectness and magnitude of effect. On the basis of "animal research reporting: *in vivo* experiment guidelines" [47] and "gold standard publication checklist" [48], we also attempted to determine the GRADE level of evidence to provide an indication of the value of this line of inquiry in the development of human studies on SCI. This information is important in allowing more precise decisions to be made in clinical settings for future research. Therefore, this meta-analysis provides up-to-date and convincing evidence of the ability of iPSC transplantation to promote locomotor recovery in rats with SCI.

Limitations

The generalizability of the results of this meta-analysis are subject to certain limitations. First, the number of publications is limited, which may influence the interpretation of the results. Thus, further relevant studies are required to validate our conclusions. Second, although the BBB score is a valid and convenient method to evaluate the neurological recovery effects in rat models after SCI, which is widely used in most publications, this method is based on subjective observations that may increase bias. Accordingly, we recommend that investigators should use the BBB score only when blinded to the intervention groups. Third, we strongly suggest that future research should focus on the other animal models to study the effects of iPSC transplantation after SCI.

Conclusion

Taken together, the results of our systematic review and meta-analyses support the hypothesis that iPSC transplantation from human fetal lung or mouse embryonic fibroblasts improves locomotor recovery in rats subjected to SCI and represents a substantially beneficial therapy. However, further studies are required to validate our conclusions and ultimately, to facilitate the development of human studies in SCI.

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Disclosure Statement

The authors declare no conflict of interests.

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