RESEARCH ARTICLE

Mapping theme trends and knowledge structures for human neural stem cells: a quantitative and co-word biclustering analysis for the 2013–2018 period

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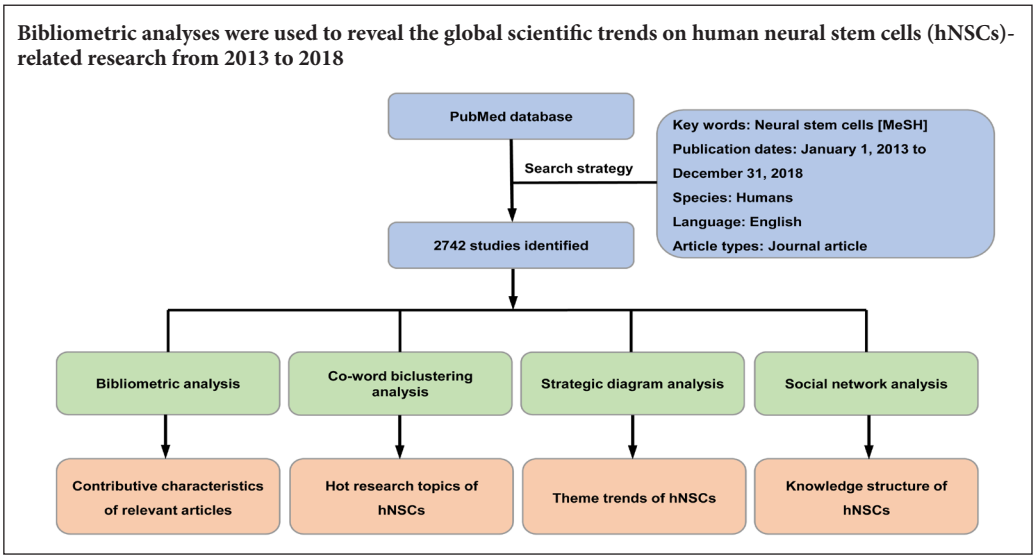
Graphical Abstract



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Abstract

Neural stem cells, which are capable of multi-potential differentiation and self-renewal, have recently been shown to have clinical potential for repairing central nervous system tissue damage. However, the theme trends and knowledge structures for human neural stem cells have not yet been studied bibliometrically. In this study, we retrieved 2742 articles from the PubMed database from 2013 to 2018 using "Neural Stem Cells" as the retrieval word. Co-word analysis was conducted to statistically quantify the characteristics and popular themes of human neural stem cell-related studies. Bibliographic data matrices were generated with the Bibliographic Item Co-Occurrence Matrix Builder. We identified 78 high-frequency Medical Subject Heading (MeSH) terms. A visual matrix was built with the repeated bisection method in gCLUTO software. A social network analysis network was generated with Ucinet 6.0 software and GraphPad Prism 5 software. The analyses demonstrated that in the 6-year period, hot topics were clustered into five categories. As suggested by the constructed strategic diagram, studies related to cytology and physiology were well-developed, whereas those related to neural stem cell applications, tissue engineering, metabolism and cell signaling, and neural stem cell pathology and virology remained immature. Neural stem cell therapy for stroke and Parkinson's disease, the genetics of microRNAs and brain neoplasms, as well as neuroprotective agents, Zika virus, Notch receptor, neural crest and embryonic stem cells were identified as emerging hot spots. These undeveloped themes and popular topics are potential points of focus for new studies on human neural stem cells.

Key Words: *nerve regeneration; human neural stem cells; PubMed; bibliometric analysis; biclustering analysis; co-word analysis; strategic diagram analysis; social network analysis; hot research topics; mapping theme trends; knowledge structures; neural regeneration*

Chinese Library Classification No. *R453; R364*

Introduction

Reynolds and Weiss isolated stem cells from the forebrain of an adult mammal in 1992 (Reynolds and Weiss, 1992), and not long after, neural stem cells (NSCs) were isolated from humans (Svendsen et al., 1998; Vescovi et al., 1999). It is now well-accepted that neurons derived from NSCs continue to form throughout adult life. Additionally, most mammals, including humans, undergo lifelong neurogenesis (Eriksson et al., 1998). NSCs can differentiate into astrocytes, neurons and oligodendrocytes, and possess an unlimited capacity for self-renewal that persists throughout the life of the animal (McKay, 1997; Seaberg and van der Kooy, 2002).

A rapid increase in stem cell research has been observed since the beginning of the 21st century. Bibliometrics has been widely used to map the literature on stem cell research. A series of bibliometric analyses of stem cell-related research and applications have been conducted for nervous system diseases such as spinal cord injury, epilepsy (Yin et al., 2012), Parkinson's disease (Li, 2012) and cerebral ischemia. More recently, researchers have endeavored to clarify the cytology, physiology, metabolism and application of human NSCs (hNSCs) (Wang et al., 2018). However, the literature on hNSCs has not yet been systematically studied. Therefore, in the present study, we used bibliometric analysis to identify the research trends associated with hNSCs. Bibliometry allows the deciphering and quantitative analysis of the hot topics in the published literature. Hot topics can be identified by techniques such as co-word analysis and co-citation analysis (Yao et al., 2014). Particularly, co-word analysis, which can estimate the relationship between two professional words in related papers, has been most commonly used (Hong et al., 2014). In this study, the extracted professional words were classified using biclustering analysis, which can cluster lines and columns simultaneously (Hartigan, 1972) and perform a partial analysis from a large amount of data. In addition, the relationships between the themes and evolutionary trends were studied by social network analysis and strategic diagram (Zhang et al., 2013). In this way, in addition to journals, countries and influential publications, we also analyzed the internal relationships, characteristics, knowledge structures and theme trends of the NSC-related literature published from 2013 to 2018. Research status and emerging issues were mapped by biclustering analysis on the basis of co-occurring MeSH terms and strategic diagram. In addition, social network analysis was performed to visualize the knowledge structure and relationships between hNSCs and their cytology, physiology, genetics and clinical applications.

Data and Methods

Data resource and search strategy

We retrieved and downloaded data from the PubMed database of the US National Center for Biotechnology Information. Medical Subject Heading (MeSH) terms, the medical vocabulary resource created by the National Library of Medicine, is the basis for Index Medicus and the MEDLINE database, and is used for indexing and cataloging articles in the PubMed system. For retrieval, articles were restricted to publication type as Journal Article, species as Humans, and language as English. "Neural Stem Cells" [MeSH] was used as the retrieval term. A total of 2742 articles published from 1 January 2013 to 31 December 2018 were retrieved. Every downloaded publication contained author, title, country, institution, MeSH terms and publication year, saved in XML format.

Data extraction and bibliographic matrix building

The bibliographic data were extracted from the PubMed database. The co-occurrence matrices and the term–source article relationships were created using Bibliographic Item Co-Occurrence Matrix Builder (Li et al., 2015). The contributive characteristics of the countries, journals, publication years and main MeSH terms/subheadings were analyzed using the Bibliographic Item Co-Occurrence Matrix Builder. In addition, the amount of high-frequency main MeSH terms/subheadings was defined by the threshold value (T), which can be expressed as: $T = (1+\sqrt[2]{1+8i})/2$, where i represents the amount of main MeSH terms/subheadings appearing just once (Donohue, 1974). Therefore, the amount of high-frequency main MeSH terms/subheadings was defined according to this expression.

Biclustering analysis of high-frequency main MeSH terms/subheadings

The high-frequency main MeSH terms/subheadings and PubMed Unique Identifiers of the hNSC-related literature retrieved from PubMed were subjected to biclustering analysis. The main MeSH terms/subtitles were classified according to the term-source article matrix.

Mountain Visualization was performed, and a visual matrix was built with the repeated bisection method in gCLUTO software (Rasmussen and Karypis, 2004). The peaks in the 3-D terrain are marked by numbers, which represent the clusters analyzed by biclustering. The color, height, volume and location of each peak in the figure were used to illustrate the data for the associated clusters. The relative location of each peak in the figure is the most informative attribute. The internal resemblance of a cluster is represented by the height of each peak. The relative resemblance of a pair of peaks is represented by the distance between them. The number of main MeSH terms/subheadings is proportional to the volume of a peak. Finally, the internal standard deviation of the objects in a cluster is shown by the peak color. Blue denotes high deviation, while red indicates low deviation. For the matrix visualization, the high-frequency main MeSH terms/subheadings are represented by the row labels, and the PubMed Unique Identifiers of source articles are indicated by the column labels, which are located on the left and top of the matrix, respectively. Based on the results of biclustering analysis, the structures of related research focuses were presented and analyzed.

Strategic diagram analysis

Based on theme centrality and density, a two-dimensional strategic diagram was built by drawing themes along two axes (Viedma-Del-Jesus et al., 2011). The external cohesion index or centrality, which indicates the central location of the subject in the frame, is represented by the X-axis. Furthermore, the internal cohesion index or density is represented by the Y-axis (Li et al., 2009). Four quadrants were generated by the two axes, and the clusters of main MeSH terms/subheadings were distributed to the four quadrants according to the results of biclustering analysis, constructing with GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA).

Social network analysis

The social network analysis network was established using Ucinet 6.0 software (Analytic Technologies, Louisville, KY, USA) according to the co-occurrence matrix of high-frequency main MeSH terms/subheadings. Subsequently, the knowledge structures in the field of hNSCs were interpreted by social network analysis. The networks of main MeSH terms/subheadings were visualized and presented in two-dimensional maps using NetDraw 2.084 software (Analytic Technologies Co.). The main MeSH terms/subheadings network was represented by nodes, with the co-occurrence frequency shown by the links. The locations of main MeSH terms/subheadings were assessed by measuring the three centralities of each node (*i.e.*, closeness, betweenness and degree) to gain an understanding on the network structure of hNSCs.

Results

Characteristics of hNSC-related publications

From 2013 to 2018, a total of 2742 papers met the search criteria mentioned above. The number of hNSC-related papers was 489 in 2013, increased to 519 in 2014, and peaked at 558 in 2016, with slight reductions in 2015 (481) and 2017 (522) (**Figure 1A**). The United States made the largest contribution to hNSC-related studies, accounting for 54.05% of the published papers. England and the Netherlands ranked second and third, respectively, in the number of papers (**Figure 1B**).

PLoS One (impact factor (IF) = 2.766, 2017) published the greatest number of hNSC-related articles (130, 4.741%), followed by *Stem Cell Reports* (73, 2.662%) (IF = 6.537, 2017) and *Scientific Reports* (61, 2.225%) (IF = 4.122, 2017). The top-ranking 15 journals are shown in **Figure 1C**.

Hot research topics based on MeSH term clusters

Among the hNSC-related literature, high-frequency main MeSH terms/subheadings had a cumulative frequency of 40.7962% (**Additional Table 1**), and were thus considered hot research topics over the past 6 years. The MeSH terms in this 6-year period were analyzed, and five clusters were identified by biclustering analysis (**Figure 2**). The mountain and matrix visualizations of the main MeSH terms/subheadings are shown in **Figure 2**. As suggested by the results, 78 high-frequency main MeSH terms/subheadings (**Additional Table 1**) were classified into five clusters. The 78 high-frequency main MeSH terms/subheadings are presented in **Figure 2** (right side), showing the terms in reference to each cluster. The hierarchical trees on the left and top represent the relationships between high-frequency main MeSH terms/subheadings and between articles, respectively. Furthermore, the representative papers in each cluster were explored by identifying and summarizing the themes. The results of cluster analysis from the high-frequency main MeSH terms/subheadings of hNSC-related studies are given in **Table 1**.

Theme trends of hNSCs

With both high density and centrality, motor themes are located in the upper right of Quadrant I. Themes with high density but inadequate external interactions are defined as specialized themes in the upper left of Quadrant II. Themes with low density and centrality are usually considered either vanishing or emerging, and are located on the left of Quadrant III. The right part of Quadrant IV contains themes with weak internal maturation but high centrality (Viedma-Del-Jesus et al., 2011). In strategic diagrams, the themes are shown as roundness in different quadrants based on centralities and densities corresponding to external and internal cohesions.

The interpretation of the strategic diagram is presented in **Figure 3A**. The clusters in Quadrant I, as central themes in the general network, are intensely connected with other clusters, with strong internal interactions (high development degree). The clusters in Quadrant II are peripheral but well developed themes, and those in Quadrant III are undeveloped and peripheral. However, the clusters in Quadrant IV are central but undeveloped, though they are slightly mature (Callon et al., 1991).

The roundness area is proportional to the amount of high-frequency main MeSH terms/subheadings for each theme cluster (**Figure 3B**). Clusters 1 and 2 in Quadrant I represent studies on NSC cytology (Cluster 1: astrocyte, oligodendroglia, neuron, neural crest and spinal cord cytology; cell culture techniques) and NSC biology (Cluster 2: cell movement and proliferation; brain, neuron, astrocyte and neurogenesis biology). These two clusters have core status and are well developed, with high density and centrality. Clusters 0 and 3 in Quadrant III represent studies on NSC pathology and virology (Cluster 0: brain neoplasm metabolism, pathology and genetics; neoplastic stem cell metabolism and pathology; Zika virus biology) and clinical applications of NSCs (Cluster 3: NSCs in Parkinson's, stroke and spinal cord injury therapy; tissue engineering; NSC proliferation and differentiation using drugs). These clusters are immature, *i.e.*, at the edge of the hNSC research field. Cluster 4 in Quadrant IV represents studies on NSC metabolism and signaling (including microRNA in neurogenesis and cell differentiation genetics; signal transduction and transcription factors). It has core status, but is undeveloped. The diagram shows the development and tendency of each hNSC theme cluster in the 6-year period examined.

Knowledge structure of hNSCs

Degree, closeness and betweenness were used to build the social network analysis network knowledge structure as centrality parameters (**Figure 4**). Furthermore, the social network analysis network was established on the basis of betweenness centrality. The node size was proportional to the betweenness centrality of main MeSH terms/subheadings, with the line thickness representing the frequency of co-occurrence.

In the network of hNSCs from 2013 to 2018, 23 main MeSH terms/subheadings (yellow and cyan circles in **Figure 4**) had a high degree of centrality, including the top 15 high-frequency ones. Notably, "Neural Stem Cells/cytolo-

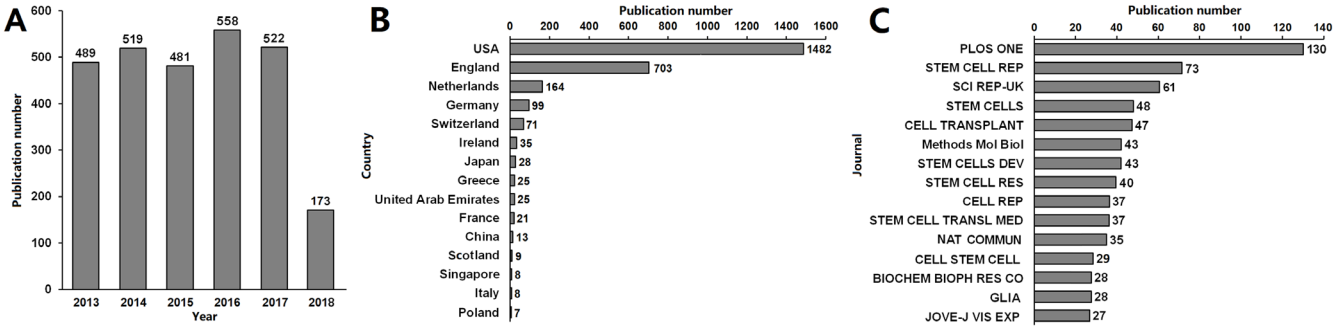

Figure 1 Characteristics of human neural stem cell-related publications.
(A) The number of publications on human neural stem cells from 2013 to 2018. (B) The number of publications from the top 15 countries. (C) The number of publications in the top 15 journals.

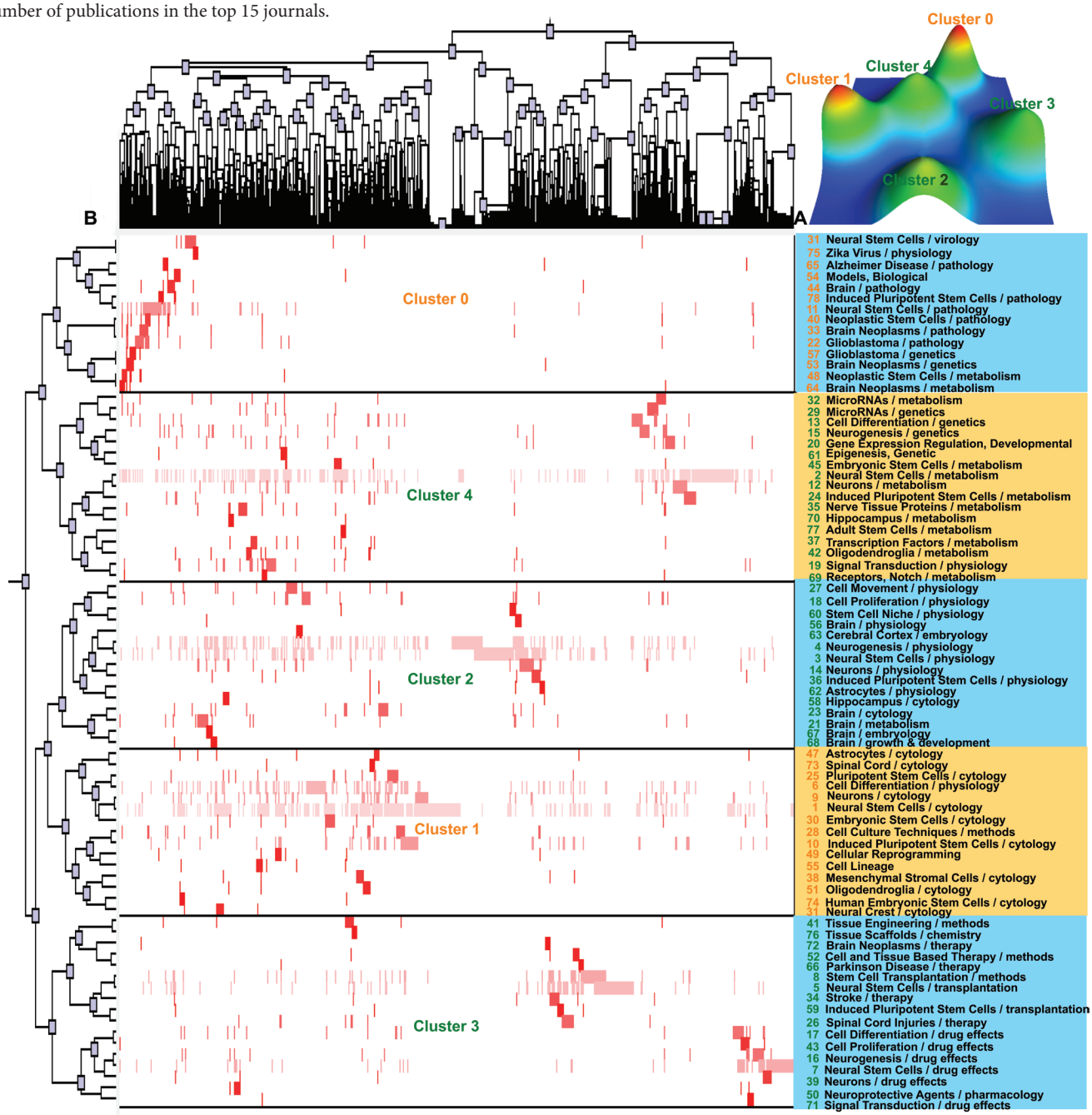

Figure 2 Biclustering analysis of 78 high-frequency main Medical Subject Heading (MeSH) terms/subheadings and articles on human neural stem cells from 2013 to 2018.
(A) Mountain visualization of biclustering of 78 high-frequency main MeSH terms/subheadings and articles. (B) Matrix visualization of biclustering of 78 high-frequency main MeSH terms/subheadings and PubMed Unique Identifiers of the articles.

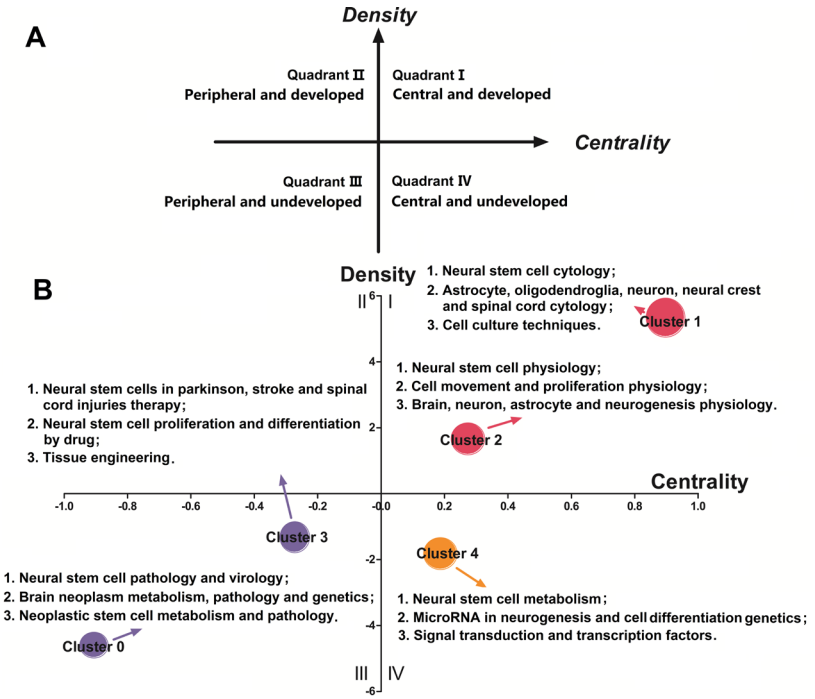

Figure 3 Strategic diagrams for human neural stem cell (hNSC)-related research.
(A) Meanings of the four quadrants in the strategic diagram. (B) Strategic diagram of hNSCs from 2013 to 2018. The size of a signal node represents the number of major Medical Subject Heading (MeSH) terms/subheadings involved in each cluster.

Table 1 Cluster analysis of high-frequency major Medical Subject Heading (MeSH) terms/subheadings of human neural stem cells

Cluster	Number of MeSH terms*	Cluster analysis
0	31,75,65,54,44,78,11,40,33,22,57,53,48,64	1. Neural stem cell pathology and virology 2. Brain neoplasm metabolism, pathology and genetics 3. Neoplastic stem cell metabolism and pathology
1	47,73,25,6,9,1,30,28,10,49,55,38,51,74,31	1. Neural stem cell cytology 2. Astrocyte, oligodendroglia, neuron, neural crest and spinal cord cytology 3. Cell culture techniques
2	27,18,60,56,63,4,3,14,36,62,58,23,21,67,68	1. Neural stem cell physiology 2. Cell movement and proliferation physiology 3. Brain, neuron, astrocyte and neurogenesis physiology
3	41,76,72,52,66,8,5,34,59,26,17,43,16,7,39,50,71	1. Neural stem cells in Parkinson, stroke and spinal cord injuries therapy 2. Neural stem cell proliferation and differentiation by drug 3. Tissue engineering
4	32,29,13,15,20,61,45,2,12,24,35,70,77,37,42,19,69	1. Neural stem cell metabolism 2. MicroRNA in neurogenesis and cell differentiation genetics 3. Signal transduction and transcription factors

*Represents the number of high-frequency major MeSH terms/subheadings shown in Additional Table 1.

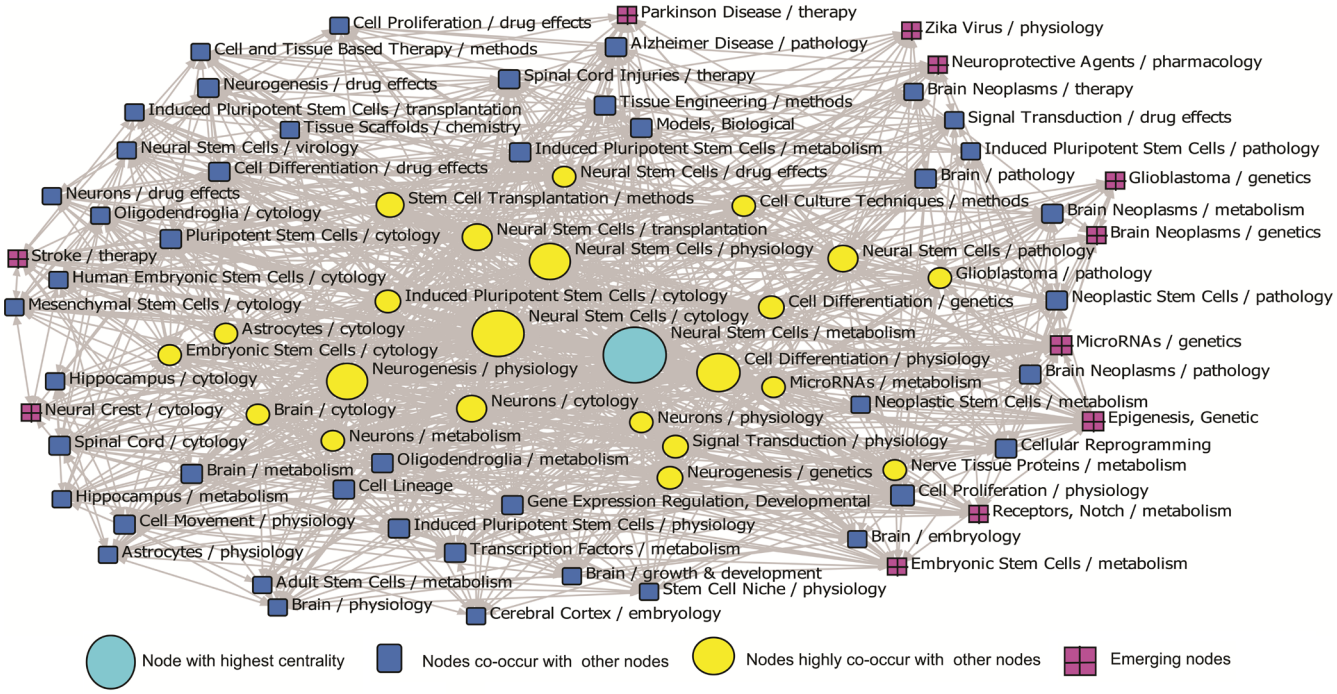

Figure 4 Social network structure map for main Medical Subject Heading (MeSH) terms/subheadings for human neural stem cell-related publications.
Social network analysis for 78 main MeSH terms/subheadings from 2013 to 2018. Nodes represent MeSH terms/subheading words, while node size and location indicate the centrality of a word in the social network analysis map. Links represent the connection between two words, and the number or thickness of the lines indicates the co-occurrence frequency of the main MeSH terms/subheading pairs.

Table 2 Individual centrality of human neural stem cells

Rank	Major MeSH terms/MeSH subheadings	Degree	Betweenness	Closeness	Rank	Major MeSH terms/MeSH subheadings	Degree	Betweenness	Closeness
1	Neural Stem Cells/cytology	1110	194.785	73	40	Neoplastic Stem Cells/pathology	79	11.251	49.333
2	Neural Stem Cells/metabolism	842	256.367	76	41	Tissue Engineering/methods	58	11.843	51.5
3	Neural Stem Cells/physiology	452	134.478	69.5	42	Oligodendroglia/metabolism	61	17.151	53
4	Neurogenesis/physiology	599	129.811	70	43	Cell Proliferation/drug effects	50	4.010	46
5	Neural Stem Cells/transplantation	323	60.345	61.5	44	Brain/pathology	54	15.211	52
6	Cell Differentiation/physiology	520	142.634	70.5	45	Embryonic Stem Cells/metabolism	53	3.258	47.5
7	Neural Stem Cells/drug effects	174	23.598	55	46	Neural Crest/cytology	45	1.530	45.5
8	Stem Cell Transplantation/methods	277	50.231	60.5	47	Astrocytes/cytology	80	24.205	53.5
9	Neurons / cytology	305	61.123	63.5	48	Neoplastic Stem Cells/metabolism	57	2.554	45.833
10	Induced Pluripotent Stem Cells/cytology	268	38.266	60	49	Cellular Reprogramming	56	15.658	52
11	Neural Stem Cells/pathology	167	62.299	59.5	50	Neuroprotective Agents/pharmacology	35	5.436	47.5
12	Neurons/metabolism	190	29.496	57	51	Oligodendroglia/cytology	65	6.441	50
13	Cell Differentiation/genetics	140	34.307	56	52	Cell and Tissue Based Therapy/methods	52	3.571	47
14	Neurons/physiology	117	21.996	54.5	53	Brain Neoplasms/genetics	43	1.630	44.5
15	Neurogenesis/genetics	116	32.762	57	54	Models, Biological	50	14.189	52
16	Neurogenesis/drug effects	81	13.072	51	55	Cell Lineage	58	18.759	52.5
17	Cell Differentiation/drug effects	123	14.689	52.5	56	Brain/physiology	52	2.727	46
18	Cell Proliferation/physiology	127	19.831	54	57	Glioblastoma/genetics	38	1.233	45
19	Signal Transduction/physiology	116	37.024	57.5	58	Hippocampus/cytology	54	3.885	47.5
20	Gene Expression Regulation, Developmental	110	16.644	54.5	59	Induced Pluripotent Stem Cells/ transplantation	45	1.570	45.5
21	Brain/metabolism	86	15.971	53	60	Stem Cell Niche/physiology	51	4.660	47.5
22	Glioblastoma/pathology	120	26.545	54.5	61	Epigenesis, Genetic	54	13.749	53
23	Brain/cytology	132	30.207	57.5	62	Astrocytes/physiology	39	4.312	46.5
24	Induced Pluripotent Stem Cells/metabolism	102	16.827	53.5	63	Cerebral Cortex/embryology	36	0.593	44.5
25	Pluripotent Stem Cells/cytology	120	18.106	54	64	Brain Neoplasms/metabolism	47	7.712	48
26	Spinal Cord Injuries/therapy	90	8.669	49.5	65	Alzheimer Disease/pathology	44	8.162	49
27	Cell Movement/physiology	103	12.382	52	66	Parkinson Disease/therapy	42	1.323	45.5
28	Cell Culture Techniques/methods	124	26.299	55	67	Brain/embryology	42	4.015	48
29	MicroRNAs/genetics	85	15.685	51.5	68	Brain/growth & development	43	2.273	47.5
30	Embryonic Stem Cells/cytology	92	22.193	55	69	Receptors, Notch/metabolism	48	5.737	48
31	Neural Stem Cells/virology	50	5.374	49	70	Hippocampus/metabolism	41	4.590	47
32	MicroRNAs/metabolism	80	24.959	55.5	71	Signal Transduction/drug effects	37	6.085	47.5
33	Brain Neoplasms/pathology	104	16.268	52	72	Brain Neoplasms/therapy	28	1.492	44
34	Stroke/therapy	70	6.512	48.5	73	Spinal Cord/cytology	51	9.501	50.5
35	Nerve Tissue Proteins/metabolism	65	27.107	55	74	Human Embryonic Stem Cells/cytology	48	6.880	50
36	Induced Pluripotent Stem Cells/physiology	61	13.069	51.5	75	Zika Virus/physiology	29	0.772	44
37	Transcription Factors/metabolism	63	11.35	52	76	Tissue Scaffolds/chemistry	38	1.342	46
38	Mesenchymal Stem Cells/cytology	61	3.810	47	77	Adult Stem Cells/metabolism	33	2.372	45
39	Neurons/drug effects	45	2.520	45.667	78	Induced Pluripotent Stem Cells/pathology	28	2.711	45.5

Table 3 Descriptive statistics for centrality measures for human neural stem cells

Centralization	Mean ± SD	Min	Max	Network centralization (%)
Degree	124.026±174.513	28.000	1110.000	12.472
Betweenness	24.769±42.672	0.593	256.367	8.020
Closeness	52.248±6.825	44.000	76.000	62.910

gy" had the highest degree of centrality (1,110; **Additional Table 1**).

The top two highest betweenness centralities were 256.367 and 194.785 (**Table 2**), corresponding to "Neural Stem Cells/metabolism" and "Neural Stem Cells/cytology", respectively. They played the most significant mediating roles in the network. Furthermore, since both terms had the highest closeness values (76 and 73, respectively), they were tightly connected with other nodes. In addition to these two main MeSH terms/subheadings, "Cell Differentiation/physiology", "Neural Stem Cells/physiology", "Neurogenesis/physiology", "Neural Stem Cells/pathology", "Neurons/cytology", as well as "Neural Stem Cells/transplantation" also had high betweenness centralities, indicative of their prominent mediating roles in the network. The mean betweenness centrality was (24.769 ± 42.672; **Table 3**). Furthermore, 11 new nodes (magenta box in **Figure 4**) at the network edge, including "Stroke/therapy", "Parkinson Disease/therapy", "Neural Crest/cytology", "MicroRNAs/genetics", "Neuroprotective Agents/pharmacology", "Glioblastoma/genetics", "Brain Neoplasms/genetics", "Epigenesis/genetics", "Receptors, Notch/metabolism", "Embryonic Stem Cells/metabolism" and "Zika Virus/physiology" were emerging hot topics of hNSC-related studies from 2013 to 2018 (**Additional Table 2**).

Discussion

NSCs in the central nervous system are capable of self-renewal and multi-potential differentiation. In recent years, NSC therapy has been used to treat many central nervous system diseases, aiming to inhibit or to reverse central nervous system damage. NSCs have been used in clinical practice mainly for direct cell transplantation therapy for central nervous system diseases, for gene delivery for gene therapy, and for modulating growth factors and cytokines for inducing self-differentiation for self-repair. These therapies have been used to treat Parkinson's disease, cerebrovascular disease, brain tumor, spinal cord injury and Alzheimer's disease (Boese et al., 2018; Ludwig et al., 2018; Mooney et al., 2018). Along with the increasing awareness of the potential clinical applications of hNSCs, hNSC-related studies have increased dramatically over the last 6 years, requiring a systematic analysis of knowledge structures and theme trends.

The PubMed database, one of the most important literature databases for the natural sciences, comprises more than 28,000,000 biomedical literature citations from journals in life science, MEDLINE and books online. In the present study, 2742 articles related to hNSCs were retrieved from the PubMed database, and the knowledge structure and progression in this field was investigated using biclustering analysis, co-word analysis, social network analysis and strategic diagram plotting. It is the first time that this method has been used to analyze the research trends of hNSCs in this 6-year period.

The United States ranked first in the number of publications on hNSC-related studies. Among the top 15 journals, it is notable that *PLoS One* has been far ahead, with 130 papers. In addition, *Stem Cell Reports* and *Scientific Reports* were the other major journals publishing hNSCs-related papers. Thus, major future developments in the field of hNSCs will likely be published by these three journals.

To methodically analyze the basic knowledge of hNSCs, we integrated social network analysis with co-word analysis. From co-word analysis, closely-related MeSH terms were grouped into clusters.

Cluster 1 is mainly related to the cytology of hNSCs (including astrocyte, oligodendroglia, neuron, neural crest and spinal cord cytology, and cell culture techniques). NSCs are generated through asymmetric division into neural precursor cells, followed by the same type of division into new functional neurons. The processes occur both in the adult central nervous system and during embryonic neural development. After isolation from primary tissues, NSCs can be cultured under nonadherent conditions in vitro, giving clonally-derived colonies (neurospheres). These cells can also be cultured as two-dimensional adherent monolayers (Adams and Morshead, 2018). NSCs can be differentiated from induced pluripotent stem cells from neurological patients as well as healthy individuals by treatment with small molecules, specific transcription factors, plasmids, microRNAs and other morphogens (Iván Velasco et al., 2014; Leonardo D'Aiuto et al., 2014). Moreover, NSCs can be produced from embryonic stem cells originating from blastocysts by treatment with extracellular matrix proteins, morphogens and other differentiation factors (Bergström and Forsberg-Nilsson, 2012). Human NSCs can be expanded in defined media containing growth factors such as basic fibroblast growth factor and epidermal growth factor, and thereafter cultured as free-floating neurospheres or monolayers (Villa et al., 2000). Li et al. (2016) reported that transduction with L-Myc (LM-NSC008) maintains the self-renewal capacity and multipotency of primary hNSCs. The immortalization with Myc was typified by long-term expansion and karyotype stability.

Cluster 2 is mainly related with the biology of hNSCs (including cell movement and proliferation, as well as brain, neuron, astrocyte and neurogenesis). In the adult mammalian brain, NSCs are located in the hippocampal subgranular zone, lateral ventricular subgranular zone and central canal of the spinal cord. These cells divide and generate new neurons in a process referred to as adult neurogenesis (Yuan et al., 2015). Although hippocampal neurogenesis is sharply attenuated with age (Sorrells et al., 2018), accumulating evidence shows that neurogenesis persists in the striatum (Ernst et al., 2018) and hippocampus (Spalding et al., 2013; Boldrini et al., 2018) in humans over their lifetime. Though neurogenesis takes place at a very low rate in healthy adult mammals, it can be stimulated by central nervous system injury (Yu et al., 2016). NSCs and neurogenic niches have been reported to exist in the central nervous system of adult mammals. Given their critical roles in health and disease, neurogenesis and gliogenesis have been studied extensively. The niche microenvironment regulates NSC survival, proliferation and differentiation under healthy and disease conditions (Pourabdolhossein et al., 2017). For example, NSC proliferation is enhanced by administration of exogenous growth factors such as ciliary neurotrophic factor, hepatocyte growth factor and epidermal growth factor (Ramírez-Castillejo et al., 2006). Clusters 1 and 2 are located in Quadrant I, and are therefore hot research topics that are well developed and centralized.

Cluster 0 is mainly associated with the pathology of NSCs and brain tumors (including brain neoplasm metabolism, pathology and genetic, neoplastic stem cell metabolism and pathology, and Zika virus biology). The stem cell-like cells in brain tumors have been identified and isolated in vitro, although whether these behaviors are associated with their *in vivo* functions remains unclear (Galli et al., 2004; Singh et al., 2004). As tumor-tropic cells, NSCs can rapidly penetrate normal organs and target metastatic and invasive tumor foci, and reach brain tumors after traversing the blood-brain barrier (Aboody et al., 2013). A first-in-human pilot safety/feasibility study of NSCs, initiated in 2010 (NCT01172964), showed that NSCs can migrate to distant tumors sites and are non-tumorigenic, thereby demonstrating the safety and ability of NSCs to target tumor foci in the brain for localized chemotherapies (Portnow et al., 2017). Clearly, every NSC source (pool or clone) must be tested for functional and genetic stabilities with increasing passage and time, together with tumor tropism, before clinical use. Notably, the alloge-

neic clonal HB1.F3.CD NSC line, which is chromosomally stable, has well-documented non-tumorigenicity (Mooney et al., 2018). Now, effective methodologies are needed to image the biodistribution of NSCs in patients. Bhagat et al. (2018) demonstrated that expression of the Zika virus envelope (E) protein leads to the accumulation of hNSCs in the G0/G1 phase of the cell cycle, thereby reducing the brain cell pool and causing microcephaly.

Cluster 3 is mainly related to the clinical applications of hNSCs (including NSCs in Parkinson's, stroke and spinal cord injure therapy, tissue engineering, and NSC proliferation and differentiation using drugs). The aim of NSC-related studies is to develop medical interventions and therapeutic strategies to recover the function and structure of the injured nervous system. As shown by pre-clinical proof-of-concept studies, NSC-based therapies provide protection against insults (Umeda et al., 2016), and can be used for neuronal replacement (Begum et al., 2015), the production of antibodies (Kanojia et al., 2015) and the targeted delivery of therapeutic agents (Aboody et al., 2013), including pro-drug-activating enzymes (Metz et al., 2013). ReNeuron has developed an hNSC-based therapy for post-stroke chronic disability on the basis of early clinical studies (clinicaltrials.gov NCT02117635). In addition, the CTX0E03 cell line from ReNeuron has been assessed in a single-center first-in-human trial of patients with moderate or severe disability 6 months to 5 years after ischemic stroke. The study revealed therapeutic potential, without safety concerns (Kalladka et al., 2016). At present, a Phase II multi-center trial of cases of upper limb disability 3–12 months after stroke is ongoing in the United Kingdom (clinicaltrials.gov NCT02117635). These studies have helped clarify the functional role of NSCs under normal and pathological conditions, as well as the challenges to their use in neural repair.

Clusters 0 and 3 in Quadrant III are existing hot topics that are undeveloped and peripheral, requiring further in-depth studies.

Cluster 4 is mainly linked to the metabolism and signaling transduction pathways of NSCs (including microRNAs in neurogenesis and cell differentiation, signal transduction and transcription factors). NSCs may integrate various homeostatic signals to modulate cellular biology on multiple levels to produce an optimal metabolic response (Knobloch and Jessberger, 2017; Ferreira et al., 2018). During neuronal differentiation, various signaling effectors, including Notch, Sonic hedgehog, bone morphogenetic proteins and Wnt, together with transcription factors such as Sox2, Nanog and Oct4, participate in regulating the pluripotentiality of NSCs (Gkikas et al., 2017; Navarro Quiroz et al., 2018). To date, over 2500 human miRNAs have been identified, and most are expressed in the brain (Shao et al., 2010). The modulating effects of microRNAs on the activities and characteristics of NSCs have been extensively studied. For example, miR-410 (Tsan et al., 2016), miR-9 (Radhakrishnan et al., 2016) and let-7a (Song et al., 2016) have been shown to be involved in NSC differentiation, and miR-184 and miR-134 have been found to participate in progenitor maintenance and the proliferation of neurons (Bian et al., 2013). Furthermore, miR-338-3p (Howe et al., 2017) and the miR-200 family (Beclin et al., 2016) are involved in neuronal maturation and neurogenesis. Connecting miRNAs with specific functions during neural development in humans will help clarify pathological and physiological processes in the central nervous system. In this study, this cluster in Quadrant IV suggests that these current hot topics are undeveloped and centralized.

Here, social network analysis showed that the top six high-frequency MeSH terms also had high degrees of centrality. MeSH terms such as "Neural Stem Cells/metabolism" had maximum direct links to other components and promoted the development of hNSC-related research. For betweenness centrality, "Neural Stem Cells/cytology", "Neural Stem Cells/metabolism", "Cell Differentiation/physiology", "Neural Stem Cells/physiology", "Neurons/cytology", "Neurogenesis/physiology", "Stem Cell Transplantation/methods" and "Induced Pluripotent Stem Cells/cytology" were located at the center of the entire network, indicating that the dominant components were most capable of regulating the co-occurrence of others. Therapy and genetics-related hot topics were along the network edge. Thus, while the cytology and physiology of hNSCs have been widely studied, NSC-related therapeutic and genetic topics are emerging research fields.

The current study is the first to comprehensively analyze hNSC-related publications using bibliometrics. Our results show that hNSCs are still in the primary development stages but have great potential for future applications. Forthcoming studies should focus on the emerging hot spots and undeveloped topics.

There are a few limitations to the present study. First, we only included journal articles, and excluded reviews and other types of literature. Because reviews were excluded, some hot research topics may be missing. Second, the articles and journals were all in English, and therefore, the exclusion of non-English papers may have affected the conclusions. Third, the co-word analysis was performed on the basis of high-frequency MeSH terms, which may have affected the results of clustering analysis, and therefore, some emerging topics may have been missed. Thus, in future studies, analyses combining new emerging topics and multiple databases should be conducted.

In summary, biclustering analysis, social network analysis and strategic diagram were performed on the basis of co-word analysis of high-frequency MeSH terms for hNSCs. Undeveloped themes, such as the application of NSCs, tissue engineering, signal transduction, microRNA, NSC pathology and virology, can be considered attractive for future studies. Furthermore, cytology, physiology, metabolism and cell signaling are core themes that have experienced sustained development from 2013 to 2018. NSC therapy for stroke and Parkinson's disease, the genetics of microRNAs, brain neoplasms and epigenesis, as well as neuroprotective agents, neural crest, embryonic stem cells, Zika virus and Notch receptor are emerging hot spots. Our findings should help provide guidance to scientists and clinicians planning

hNSC-related research projects.

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Additional files:
Additional file 1: *Open peer review report 1.*
Additional Table 1: *High-frequency MeSH terms/MeSH subheadings from the included articles on hNSCs.*
Additional Table 2: *Sub-categories of emerging hot topics.*

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Additional Table 1 High-frequency MeSH terms/MeSH subheadings from the included articles on human neural stem cells

Rank.	Major MeSH terms/MeSH subheadings	Frequency	Proportion of frequency (%)	Cumulative percentage (%)
1	Neural Stem Cells/cytology	644	4.8744	4.8744
2	Neural Stem Cells/metabolism	551	4.1705	9.0448
3	Neural Stem Cells/physiology	351	2.6567	11.7015
4	Neurogenesis/physiology	336	2.5431	14.2446
5	Neural Stem Cells/transplantation	259	1.9603	16.2050
6	Cell Differentiation/physiology	226	1.7106	17.9155
7	Neural Stem Cells/drug effects	177	1.3397	19.2552
8	Stem Cell Transplantation/methods	163	1.2337	20.4889
9	Neurons/cytology	123	0.9310	21.4199
10	Induced Pluripotent Stem Cells/cytology	122	0.9234	22.3433
11	Neural Stem Cells/pathology	98	0.7417	23.0851
12	Neurons/metabolism	90	0.6812	23.7663
13	Cell Differentiation/genetics	67	0.5071	24.2734
14	Neurons/physiology	66	0.4995	24.7729
15	Neurogenesis/genetics	63	0.4768	25.2498
16	Neurogenesis/drug effects	62	0.4693	25.7190
17	Cell Differentiation/drug effects	61	0.4617	26.1807
18	Cell Proliferation/physiology	57	0.4314	26.6122
19	Signal Transduction/physiology	57	0.4314	27.0436
20	Gene Expression Regulation, Developmental	55	0.4163	27.4599
21	Brain/metabolism	54	0.4087	27.8686
22	Glioblastoma/pathology	54	0.4087	28.2773
23	Brain/cytology	52	0.3936	28.6709
24	Induced Pluripotent Stem Cells/metabolism	52	0.3936	29.0645
25	Pluripotent Stem Cells/cytology	52	0.3936	29.4581
26	Spinal Cord Injuries/therapy	51	0.3860	29.8441
27	Cell Movement/physiology	51	0.3860	30.2301
28	Cell Culture Techniques/methods	49	0.3709	30.6010
29	MicroRNAs/genetics	45	0.3406	30.9416
30	Embryonic Stem Cells/cytology	45	0.3406	31.2822
31	Neural Stem Cells/virology	44	0.3330	31.6152
32	MicroRNAs/metabolism	41	0.3103	31.9255
33	Brain Neoplasms/pathology	40	0.3028	32.2283
34	Stroke/therapy	39	0.2952	32.5235
35	Nerve Tissue Proteins/metabolism	36	0.2725	32.7959
36	Induced Pluripotent Stem Cells/physiology	35	0.2649	33.0609
37	Transcription Factors/metabolism	33	0.2498	33.3106
38	Mesenchymal Stem Cells/cytology	32	0.2422	33.5528

39	Neurons/drug effects	32	0.2422	33.7950
40	Neoplastic Stem Cells/pathology	32	0.2422	34.0372
41	Tissue Engineering/methods	30	0.2271	34.2643
42	Oligodendroglia/metabolism	29	0.2195	34.4838
43	Cell Proliferation/drug effects	29	0.2195	34.7033
44	Brain/pathology	29	0.2195	34.9228
45	Embryonic Stem Cells/metabolism	28	0.2119	35.1347
46	Neural Crest/cytology	27	0.2044	35.3391
47	Astrocytes/cytology	27	0.2044	35.5434
48	Neoplastic Stem Cells/metabolism	27	0.2044	35.7478
49	Cellular Reprogramming	26	0.1968	35.9446
50	Neuroprotective Agents/pharmacology	26	0.1968	36.1414
51	Oligodendroglia/cytology	26	0.1968	36.3382
52	Cell and Tissue Based Therapy/methods	25	0.1892	36.5274
53	Brain Neoplasms/genetics	25	0.1892	36.7166
54	Models, Biological	25	0.1892	36.9058
55	Cell Lineage	25	0.1892	37.0951
56	Brain/physiology	25	0.1892	37.2843
57	Glioblastoma/genetics	24	0.1817	37.4659
58	Hippocampus/cytology	24	0.1817	37.6476
59	Induced Pluripotent Stem Cells/transplantation	24	0.1817	37.8292
60	Stem Cell Niche/physiology	23	0.1741	38.0033
61	Epigenesis, Genetic	23	0.1741	38.1774
62	Astrocytes/physiology	22	0.1665	38.3439
63	Cerebral Cortex/embryology	22	0.1665	38.5104
64	Brain Neoplasms/metabolism	22	0.1665	38.6770
65	Alzheimer Disease/pathology	22	0.1665	38.8435
66	Parkinson Disease/therapy	21	0.1589	39.0024
67	Brain/embryology	21	0.1589	39.1614
68	Brain/growth & development	21	0.1589	39.3203
69	Receptors, Notch/metabolism	20	0.1514	39.4717
70	Hippocampus/metabolism	20	0.1514	39.6231
71	Signal Transduction/drug effects	20	0.1514	39.7744
72	Brain Neoplasms/therapy	20	0.1514	39.9258
73	Spinal Cord/cytology	20	0.1514	40.0772
74	Human Embryonic Stem Cells/cytology	19	0.1438	40.2210
75	Zika Virus/physiology	19	0.1438	40.3648
76	Tissue Scaffolds/chemistry	19	0.1438	40.5086
77	Adult Stem Cells/metabolism	19	0.1438	40.6524
78	Induced Pluripotent Stem Cells/pathology	19	0.1438	40.7962

Additional Table 2 Sub-categories of emerging hot topics

No.	Emerging hot topics	Sub-categories	Characteristics
1	Zika Virus/physiology	Cluster 0	Peripheral and undeveloped
2	Neural Crest/cytology	Cluster 1	Central and developed
3	Stroke/therapy	Cluster 3	Peripheral and undeveloped
4	Parkinson Disease/therapy	Cluster 3	Peripheral and undeveloped
5	Neuroprotective Agents/pharmacology	Cluster 3	Peripheral and undeveloped
6	MicroRNAs/genetics	Cluster 4	Central and undeveloped
7	Glioblastoma/ genetics	Cluster 4	Central and undeveloped
8	Brain Neoplasms/ genetics	Cluster 4	Central and undeveloped
9	Epigenesis/ genetics	Cluster 4	Central and undeveloped
10	Receptors, Notch/metabolism	Cluster 4	Central and undeveloped
11	Embryonic Stem Cells/ metabolism	Cluster 4	Central and undeveloped