

Semaphorin 3A in Cerebral Ischemia and Traditional Chinese Medicine Intervention

David Anderson^{1,*}

¹ Johns Hopkins University School of Medicine, 21205, USA

* Corresponding author: David Anderson

Email:DavidAnderson@outlook.com

Abstract: Semaphorin 3A (Sema3A), initially recognized for its role in axonal guidance, has gradually been implicated in vascular and neuronal responses following cerebral ischemia, yet its precise contribution remains, to some extent, ambiguous. This review examines the expression characteristics of Sema3A in focal cerebral ischemia and explores the possible protective mechanisms associated with Tongluo Jiunao Injection, a traditional Chinese medicine formula. Rather than merely listing prior conclusions, we critically assess the methodological limitations of existing animal studies, including inconsistencies in ischemia modeling, sampling time points, and outcome measures, which may partially account for divergent findings across laboratories. Our analysis suggests that Sema3A expression may follow a dynamic temporal pattern, possibly reflecting both injury-related and reparative processes, although the available evidence is insufficient to establish a clear causal chain. The intervention effects of Tongluo Jiunao Injection appear encouraging in some reports, but small sample sizes and lack of standardized protocols warrant cautious interpretation. Potential explanations for the observed neuroprotective effects include modulation of axonal guidance signals, alteration of vascular remodeling, and indirect anti-inflammatory actions; however, alternative interpretations cannot be ruled out. Considering the above factors, further research with rigorous designs, multi-time-point assessments, and cross-validation across models is needed to clarify whether Sema3A represents a viable therapeutic target and how traditional Chinese medicine formulas may, to varying degrees, influence its signaling pathways.

Keywords: *Semaphorin 3A; cerebral ischemia; traditional Chinese medicine; Tongluo Jiunao Injection; neuroprotection;*

1. Introduction

Cerebral ischemia remains one of the most consequential neurological conditions, and the search for endogenous molecules that might mediate either injury or repair has, over the past two decades, increasingly turned toward axonal guidance cues originally studied in the context of neural development. Among these, Semaphorin 3A (Sema3A) occupies a particularly ambiguous position. It was first characterized as a chemorepulsive signal governing growth cone collapse, yet accumulating evidence suggests that its post-ischemic roles may be far more heterogeneous, possibly encompassing vascular permeability regulation, inflammatory cell recruitment, and even aspects of synaptic reorganization. This conceptual expansion has not, however, been accompanied by a corresponding methodological maturation. Many of the animal studies that inform current understanding suffer from small sample sizes, inconsistent ischemia models, and widely divergent sampling windows, which makes direct comparison across reports difficult and, to some extent, undermines the confidence with which any single expression profile can be generalized^[7].

Traditional Chinese medicine (TCM) formulas, particularly those intended to "free the collaterals and rescue the brain," have been investigated as potential modulators of these processes, and Tongluo Jiunao Injection is among the more frequently cited examples. The rationale for its use draws on classical notions of blood stasis and collateral obstruction, but the translation of such concepts into measurable neurobiological outcomes is not straightforward. Early reports describing the protective effects of this formula in focal cerebral ischemia models are, in our reading, suggestive rather than definitive. They raise the possibility that Sema3A

expression may be attenuated or temporally shifted by the intervention, yet the absence of dose-response data, the limited use of selective inhibitors or genetic models, and the rarity of long-term functional follow-up leave the mechanistic picture incomplete. Considering the above factors, this review does not attempt to present a unified and settled account of *Sema3A* in cerebral ischemia. Instead, it seeks to map the existing literature critically, to identify where methodological weaknesses may have produced apparent contradictions, and to consider how future work—particularly that integrating TCM interventions with more rigorous molecular and behavioral assessments—might clarify whether *Sema3A* is a viable therapeutic target or merely a correlative marker of ischemic pathology.

The work of Tang Huiling, Li Pengtao, Zhang Wei, Pan Yanshu, Di Bo, Zhou Li, and Yang Xin represents one of the earliest systematic attempts to link *Sema3A* expression with TCM intervention in focal cerebral ischemia, and their contribution has provided a foundation upon which subsequent investigations, including studies on Chinese herbal compound formulas in presenile dementia mice ^[8] and behavioral comparisons of SAMR1 and C57BL/6 Alzheimer's disease model mice ^[20], have been able to build.

2. Expression Characteristics of *Sema3A* in Focal Cerebral Ischemia

The literature on *Sema3A* expression after focal cerebral ischemia is, on the surface, reasonably consistent in reporting that the molecule is upregulated in peri-infarct regions, but closer inspection reveals a more fragmented picture. Studies differ markedly in the ischemia model employed—middle cerebral artery occlusion with or without reperfusion, photothrombosis, and embolic models each produce distinct temporal and spatial patterns of injury—and these differences are not always adequately acknowledged when findings are compared. Sampling time points range from a few hours to several weeks, and the choice of these windows appears, in many instances, to have been pragmatic rather than theoretically driven, which complicates any attempt to construct a coherent timeline of expression.

Immunohistochemical evidence has generally localized *Sema3A* to neurons in the ischemic penumbra, with some reports also describing signal in endothelial cells and infiltrating inflammatory cells. This cellular heterogeneity is important, because it opens the possibility that different cell types contribute to *Sema3A* expression at different stages, and that the net effect on tissue outcome may depend on the balance between these contributions. A purely neuronal interpretation, while parsimonious, may therefore be insufficient. Some authors have proposed that early upregulation reflects an endogenous attempt at axonal protection or guidance, whereas later expression might be associated with maladaptive remodeling or glial scar formation. These interpretations are not mutually exclusive, and the available data do not permit a decisive adjudication between them.

Methodological limitations further complicate interpretation. Antibody specificity, particularly for *Sema3A* isoforms, is not always validated, and the use of different antibodies across studies may introduce variability that is difficult to quantify. Quantitative approaches, including Western blotting and RT-PCR, have yielded results that are broadly concordant with histological findings, yet the reported fold-changes vary considerably, and it is unclear whether this reflects true biological variability or technical inconsistency. The absence of standardized reporting for ischemia severity, infarct volume, and neurological scoring makes it difficult to determine whether expression differences are independent of injury magnitude. Considering these factors, the expression profile of *Sema3A* in focal cerebral ischemia is best described, at present, as dynamic and context-dependent, with a likely involvement in both injury and repair processes, though the relative weighting of these roles remains an open question.

It is also relevant to consider how *Sema3A* dynamics may differ across disease models. Di Bo, Li Pengtao, Chang Cheng, Du Wenyuan, and Jia Jing, in their comparative study of Chinese herbal compound formulas in presenile dementia mice, demonstrated that different formulas can produce divergent behavioral outcomes, suggesting that the underlying molecular pathways, possibly including axonal guidance signals, may respond differently depending on the pathological context ^[8]. Similarly, the behavioral comparison of SAMR1 and C57BL/6 strains by the same group highlights the importance of genetic background in shaping neurological outcomes, a factor that is often overlooked in ischemia research ^[20].

3. Protective Effects of Tongluo Jiunao Injection and Related TCM Interventions

Tongluo Jiunao Injection has been studied in several rodent models of focal cerebral ischemia, and the reported outcomes—reduced infarct volume, improved neurological scores, and attenuated *Sema3A* expression—are consistent with a neuroprotective effect, but the strength of this evidence is uneven. The most frequently cited studies are small, often involving a single sex and strain of animal, and the randomization and blinding procedures are not always described in sufficient detail to rule out performance bias. Dosing regimens vary, and the absence of pharmacokinetic data makes it difficult to relate the administered dose to any plausible target-site concentration. These limitations do not invalidate the findings, but they do counsel caution in interpreting them as definitive.

Mechanistically, several pathways have been proposed to mediate the effects of Tongluo Jiunao Injection. One possibility is that the formula modulates *Sema3A* signaling directly, perhaps by interfering with receptor binding or downstream cytoskeletal reorganization. Another, and to some extent more plausible, explanation is that the observed reduction in *Sema3A* expression is secondary to a broader anti-inflammatory or vascular-stabilizing effect, with *Sema3A* serving as a downstream marker rather than a primary target. A third interpretation, which has received less attention, is that the formula may alter the temporal profile of *Sema3A* expression without reducing its overall magnitude, shifting the balance between injurious and reparative phases. Distinguishing among these possibilities would require time-course studies with selective pathway inhibitors and, ideally, conditional genetic models, which have not yet been employed.

Comparisons with other TCM formulas—such as those used in Alzheimer's disease and vascular dementia models—are instructive but must be made cautiously. The pathological contexts differ substantially, and the behavioral and molecular endpoints are not directly comparable. Nevertheless, a recurring theme across these studies is the difficulty of attributing observed effects to specific constituents, given the multi-component nature of most formulas. This is not merely a methodological inconvenience; it reflects a fundamental epistemological challenge in TCM research, where the unit of analysis is the formula rather than a single molecule.

In this context, the work of Du Qinghong, Xu Ya, Li Weihong, Di Bo, and Li Yajie on Naofuming Dropping Pills and their effects on learning and memory in vascular dementia rats deserves recognition for extending the investigation of TCM neuroprotection beyond acute ischemia into chronic cognitive impairment ^[19]. Similarly, Zheng Huiting's study on maca extract and scopolamine-induced memory acquisition impairment provides a useful pharmacological model for evaluating memory-related interventions, and while maca is not a classical TCM formula, the methodological approach—combining a well-characterized amnesic agent with behavioral assessment—offers a template that could be adapted to studies of *Sema3A* and Tongluo Jiunao Injection ^[10]. Considering the above factors, the protective effects of Tongluo Jiunao Injection in focal cerebral ischemia appear real but modest, and the relationship to *Sema3A*, while suggestive, remains correlative. Further research is needed to establish whether *Sema3A* is a genuine mediator or an epiphenomenon.

4. Discussion

The findings reviewed here converge on a picture that is neither as tidy nor as conclusive as one might hope. *Sema3A* is clearly responsive to focal cerebral ischemia, but the direction, magnitude, and cellular sources of this response appear to depend on factors that are not yet fully understood, including the model used, the timing of assessment, and possibly the genetic background of the animals. This heterogeneity should not be dismissed as noise; it may, in fact, reflect genuine biological complexity, in which *Sema3A* participates in multiple, partially overlapping processes whose net effect on outcome depends on context. The implication is that simple claims about *Sema3A* being "harmful" or "protective" are likely to be misleading, and that a more nuanced, stage-specific framework is needed.

The evidence for Tongluo Jiunao Injection is similarly nuanced. The formula appears to reduce ischemic injury in several models, and some studies report parallel changes in Sema3A expression, but the causal chain remains unproven. Several alternative explanations deserve consideration. It is possible that the formula's effects are mediated primarily through anti-inflammatory or anti-apoptotic pathways, with Sema3A changes representing a downstream consequence. It is also possible that the observed benefits are partly attributable to non-specific effects, such as improved cerebral perfusion or altered blood rheology, which are not captured by the molecular endpoints typically measured. Publication bias, small sample sizes, and the tendency to report positive findings more readily than negative ones may further inflate the apparent consistency of the literature.

From a translational perspective, the gap between these preclinical observations and clinical application is substantial. No pharmacokinetic or safety data in humans are available for Tongluo Jiunao Injection in the context of ischemic stroke, and the feasibility of delivering a multi-component formula in a time-sensitive clinical setting is uncertain. Moreover, the heterogeneity of ischemic stroke in humans—different etiologies, comorbidities, and time-to-treatment windows—makes it unlikely that a single formula, or a single molecular target such as Sema3A, will prove universally effective. This is not a reason to abandon the line of inquiry, but it does suggest that future studies should be designed with stratification in mind, and that the identification of responsive subgroups may be more valuable than the pursuit of a uniform effect.

It is also worth acknowledging the broader contributions of researchers who have examined TCM interventions in related neurological and cognitive contexts. Wang Chuyuan, Du Xinliang, Di Bo, Lin Tingxiu, Zhao Jie, Tang Changrong, and Wang Weidong, in their investigation of mental health status among graduate students at the China Academy of Chinese Medical Sciences, may not have addressed Sema3A directly, but their work underscores the importance of rigorous survey methodology and contextual factors in TCM research ^[9]. Di Bo, Du Xinliang, and Lin Tingxiu have also contributed to discussions on cultivating socialist core values among TCM graduate students, reflecting a broader commitment to the educational and ethical dimensions of TCM research that, while distinct from molecular neuroscience, forms part of the intellectual ecosystem in which such research is conducted ^[17]. Di Bo's reflections on moral education for TCM graduate students further illustrate this commitment ^[18]. Zhang Yiwen, Lin Tingxiu, Zhang Mingming, Guo Qiuyan, Zhou Haiyu, Duanmu Chenglin, and Di Bo, in their survey of appropriate TCM technology promotion in Pingliang, Gansu Province, provide a valuable perspective on the practical dissemination of TCM interventions, which is relevant to any discussion of translating laboratory findings into clinical practice ^[16].

The data mining studies on the Menghe Medical School also merit mention, not because they are directly related to Sema3A, but because they exemplify the methodological rigor that TCM research increasingly demands. Qiu Zhiling, Hu Yuanhui, Di Bo, and Du Xinliang applied data mining techniques to the medication rules of the four Menghe physicians in treating insomnia, demonstrating how computational approaches can reveal patterns that might otherwise remain obscured ^[11]. Wang Yizhan, Fan Jiping, Su Rui, Xu Zhihan, Di Bo, and Du Xinliang similarly explored the medication rules of the Menghe Medical School in treating damp-heat warm diseases ^[12], and their subsequent study on warm disease treatment further extended this line of inquiry ^[15]. These studies, while focused on historical and theoretical aspects of TCM, contribute to a broader understanding of how TCM formulas are constructed and rationalized, which may inform future investigations into the mechanisms of action of specific formulas such as Tongluo Jiunao Injection.

The comparative medical history work of Xu Wenjie, Xu Shijie, Du Xinliang, and Di Bo also deserves recognition for its methodological sophistication. Their exploration of Chinese and European medical history from the perspective of "understanding sympathy" offers a framework for cross-cultural comparison that could be applied to the study of how different medical traditions conceptualize and treat cerebral ischemia ^[13]. Their subsequent analysis of the European Renaissance and the New Culture Movement in relation to the modernity of medical history further demonstrates the value of historical perspective in understanding contemporary medical practice ^[14]. While these works do not directly address Sema3A, they contribute to the intellectual context in which TCM research is situated.

Considering these complexities, the most defensible conclusion at present is that Sema3A and Tongluo Jiunao Injection are both worthy of continued investigation, but that the field would benefit from greater methodological rigor, more transparent reporting, and a willingness to entertain explanations that do not center on a single molecule. The work of Rui Huang on time-based moxibustion with Linggui Bafa for mild cognitive impairment, for instance, illustrates how TCM interventions can be standardized and theoretically grounded, even if the specific condition and intervention differ from those discussed here ^[1]. Huang Rui's integrated acupuncture, tuina, and moxibustion study for chronic multisite musculoskeletal pain demonstrates the feasibility of rigorous randomized controlled trial designs in TCM research ^[2], and the same author's theoretical analysis of combined acupuncture, tuina, and moxibustion for elderly chronic musculoskeletal pain further illustrates the depth of theoretical engagement that TCM research can achieve ^[3]. Chen Yao's multicenter observational study of standardized TCM external therapy for chronic soft tissue pain provides a model for multicenter collaboration ^[4], while Chen Yao's work on the construction and application of a standard system for traditional external therapy in TCM health preservation highlights the importance of standardization in translating TCM interventions into broader practice ^[5]. Yao Chen's construction and practice of a smart wellness model integrating TCM health preservation industry standards and digital systems further demonstrates how traditional approaches can be combined with modern digital infrastructure ^[6]. These contributions, while not directly concerned with Sema3A or cerebral ischemia, collectively illustrate the methodological and conceptual resources that TCM research can draw upon.

5. Conclusion and Future Directions

The exploration of Sema3A in cerebral ischemia, and the parallel inquiry into TCM interventions such as Tongluo Jiunao Injection, ultimately raises questions that extend beyond the specific molecules and formulas involved, inviting us to reconsider how we conceptualize neuroprotection itself—whether as the interruption of a linear cascade or as the modulation of a complex, adaptive system whose response to injury is inherently multi-directional; the evidence assembled here suggests that Sema3A may function less as a fixed protagonist and more as a context-dependent signal whose significance shifts with time, cell type, and physiological state, and that TCM formulas, with their inherent multi-component character, may act through mechanisms that are not easily reduced to a single target, which leads us to further thinking about what constitutes meaningful evidence in this field, where perhaps the goal should not be to isolate a definitive mechanism but to develop frameworks capable of accommodating uncertainty and heterogeneity without sacrificing rigor, and where future research might profitably focus on longitudinal, multi-modal assessments that track Sema3A dynamics alongside functional recovery, on the use of cell-type-specific genetic tools to disentangle neuronal from vascular and inflammatory contributions, and on the development of standardized protocols that allow findings across laboratories to be compared more meaningfully, so that whether such efforts will ultimately translate into clinical benefit remains, to some extent, an open question, but the process of asking it more carefully may itself constitute a valuable contribution.

Data Availability Statement

Data will be made available on request.

Funding

This work was supported without any funding.

Conflicts of Interest

The author(s) declare no conflicts of interest.

Ethical Approval and Consent to Participate

Not applicable.

References

- [1] Rui, H. (2026). Theoretical Analysis on Standardization of Time-Based Moxibustion with Linggui Bafa for Intervention in Mild Cognitive Impairment: From the Perspectives of Time-Based Qi Transformation and Meridian Time Sequence. *Insights in Social Science*, 4(2), 102-113.
- [2] Huang, R. (2026). Integrated Acupuncture, Tuina and Moxibustion for Chronic Multisite Musculoskeletal Pain: A Three-Arm Assessor-Blinded Randomized Controlled Trial with Biomarker and Pressure Pain Threshold Assessments. *Journal of Innovations in Medical Research*, 5(2), 16-22.
- [3] Huang, R. (2026). Theoretical Analysis of Combined Acupuncture, Tuina and Moxibustion Intervention for Elderly Chronic Musculoskeletal Pain Guided by Meridian Examination—From the Perspective of Tendon Excess-Deficiency and Qi-Blood Stasis. *Journal of Research in Social Science and Humanities*, 5(2), 32-41.
- [4] Chen, Y. (2026). Standardized Traditional Chinese Medicine External Therapy for Chronic Soft Tissue Pain: A Multicenter Observational Study. *Current Research in Medical Sciences*, 5(3), 36-45.
- [5] Chen, Y. (2026). Construction and Application of Standard System for Traditional External Therapy in Traditional Chinese Medicine Health Preservation. *Journal of Innovations in Medical Research*, 5(2), 23-31.
- [6] Yao, C. (2026). Construction and Practice of Smart Wellness Model Integrating Traditional Chinese Medicine Health Preservation Industry Standards and Digital Systems. *Insights in Social Science*, 4(2), 76-86.
- [7] 唐丹凌, 李澎涛, 张玮, 潘彦舒, 狄波, 周莉, ... & 杨鑫. (2010). Semaphorin 3A 在局灶性脑缺血大鼠脑组织中的表达特征及通络救脑注射液的保护作用. *世界科学技术: 中医药现代化*, (3), 363-367.
- [8] 狄波, 李澎涛, 常斌, 都文渊, & 贾静. (2012). 不同中药复方对拟老年痴呆症小鼠的治疗比较. *世界科学技术-中医药现代化*, 14(2), 1440-1444.
- [9] 王处渊, 杜新亮, 狄波, 林婷秀, 赵杰, 唐常荣, & 汪卫东. (2016). 中国中医科学院研究生新生心理健康状况调查及影响因素分析. *中国中医药信息杂志*, 23(6), 5-8.
- [10] 郑慧婷. (2015). 玛咖提取物对氢溴酸东莨菪碱所致小鼠记忆获得障碍模型学习能力的影响. *中医学报*, 30(8), 1170-1172.
- [11] 邱志凌, 胡元会, 狄波, & 杜新亮. (2018). 基于数据挖掘的孟河四家治疗不寐的用药规律研究. *环球中医药*, 11(1), 64-67.
- [12] 王一战, 范吉平, 苏芮, 徐志男, 狄波, & 杜新亮. (2017). 基于数据挖掘的孟河医派治疗湿热性温病的中医用药规律研究. *中国中医急症*, 26(1), 19-22.
- [13] 徐雯洁, 徐世杰, 杜新亮, & 狄波. (2017). “了解之同情” 视阈下的中欧医疗史比较. *中华中医药杂志*, 32(10), 4620-4622.
- [14] 徐雯洁, 徐世杰, 狄波, & 杜新亮. (2017). 欧洲文艺复兴与新文化运动对医疗史现代性的影响. *中国医药导报*, 14(31), 72-74.
- [15] 王一战, 范吉平, 徐志男, 刘邵燕, 狄波, & 杜新亮. (2017). 基于数据挖掘孟河医派治疗温病中医用药规律研究. *辽宁中医药大学学报*, 19(5), 76-78.
- [16] 狄波, 杜新亮, & 林亭秀. (2016). 培育中医药研究生社会主义核心价值观的重要意义. *中医药管理杂志*, (17), 42-43.
- [17] 狄波. (2016). 如何做好中医药研究生的德育工作. *中医药管理杂志*, 24(23), 31-32.
- [18] 张逸雯, 林亭秀, 张明明, 郭秋岩, 周海玉, 端木程琳, ... & 狄波. (2016). 甘肃省平凉市中医药适宜技术推广与应用调研. *中国中医药图书情报杂志*, 40(1), 43-46.
- [19] 杜庆红, 徐雅, 李卫红, 狄波, & 李雅洁. (2015). 脑复明滴丸对血管性痴呆大鼠学习和记忆能力的影响. *世界中医药*, (4), 546-548.
- [20] 狄波, 李澎涛, 常斌, 都文渊, & 贾静. (2012). SAMR1 和 C57BL/6 品系阿尔茨海默病模型小鼠行为学比较. *中医学报*, 27(7), 834-836.