Review / Derleme

Ege Journal of Medicine / *Ege Tıp Dergisi* 2025; 64 (2): 415-423

Cancer stem cells and the SHH pathway in malignant melanoma: Therapeutic implications

Malign melanomda kanser kök hücreleri ve SHH yolağı: Terapötik çıkarımlar

Berrin Ozdil[1,2] Huseyin Aktug[3]

[1]Department of Physics, Faculty of Science, Izmir Institute of Technology, Izmir, Türkiye

[2]Department of Histology and Embryology, Faculty of Medicine, Suleyman Demirel University, Isparta, Türkiye

[3] Department of Histology and Embryology, Faculty of Medicine, Ege University, Izmir, Türkiye

ABSTRACT

Melanoma is an aggressive skin cancer arising from melanocytes. Melanoma is a complex disease, sourced from genetic and environmental factors. Within melanoma, cancer stem cells (CSCs) play a crucial role in tumor progression, therapeutic resistance, and recurrence due to their capabilities for self-renewal and differentiation. The Sonic Hedgehog (SHH) signaling pathway is an important regulator of CSCs and is essential for cell differentiation and proliferation. Due to the known role in embryonic development and involvement in cancers, SHH pathway significantly affects CSC behavior in malignant melanoma, promoting tumorigenicity, metastasis, and resistance to therapies. This pathway coordinates canonical mechanisms involving Gli transcription factors and non-canonical mechanisms affecting cell migration and cytoskeletal organization. Targeting the SHH pathway has emerged as a promising therapeutic strategy, with inhibitors focusing on components like Smoothened (Smo) and Gli proteins. However, resistance to these inhibitors necessitates further exploration of novel therapeutic combinations. Current research focuses on combining SHH inhibitors with immunotherapies for more effective, long-lasting responses. Targeted medicines, which disrupt SHH processes, attempt to eliminate the fundamental causes of carcinogenesis and increase melanoma patient survival rates.

Keywords: Melanoma, Cancer Stem Cells (CSC), Sonic Hedgehog (SHH) Signaling Pathway, Therapeutic Resistance, Metastasis

ÖZ

Melanom, melanositlerden kaynaklanan agresif bir cilt kanseridir. Melanom hem genetik hem de çevresel faktörlerden kaynaklanan karmaşık bir hastalıktır. Melanomda, kanser kök hücreleri (CSC'ler), kendini yenileme ve farklılaşma yetenekleri nedeniyle tümör ilerlemesinde, terapötik dirençte ve nüksde önemli bir rol oynar. Sonic Hedgehog (SHH) sinyal yolağı CSC'lerin önemli bir düzenleyicisidir ve hücre farklılaşması ve çoğalması için gereklidir. Embriyonik gelişimdeki ve kanserlerdeki rolü bilindiğinden, SHH yolağı melanomda CSC davranışını önemli ölçüde etkileyerek tümörijenisiteyi, metastazı ve tedavilere direnci teşvik eder. Bu yolak, Gli transkripsiyon faktörlerini içeren kanonik mekanizmaları ve hücre göçünü ve hücre iskeleti organizasyonunu etkileyen kanonik olmayan mekanizmaları koordine eder.

Corresponding author: Huseyin Aktug

Department of Histology and Embryology, Faculty of Medicine, Ege University, Izmir, Türkiye

E-mail: *huseyin.aktug@ege.edu.tr*

Application date: 24.09.2024 Accepted: 27.01.2025

SHH yolağının hedeflenmesi, Smoothened (Smo) ve Gli proteinleri gibi bileşenlere odaklanan inhibitörlerle umut verici bir terapötik strateji olarak ortaya çıkmıştır. Bununla birlikte, bu inhibitörlere karşı direnç, yeni terapötik kombinasyonların daha fazla araştırılmasını gerektirmektedir. Mevcut araştırmalar, daha etkili ve uzun süreli yanıtlar için SHH inhibitörlerini immünoterapilerle birleştirmeye odaklanmaktadır. SHH süreçlerini bozan hedefe yönelik ilaçlar, karsinogenezin temel nedenlerini ortadan kaldırmaya ve melanom hastalarının hayatta kalma oranlarını artırmaya çalışmaktadır.

Anahtar Sözcükler: *Malign Melanom, Kanser Kök Hücreleri (CSC), Sonic Hedgehog (SHH) Sinyal Yolağı, Terapötik Direnç, Metastaz*

INTRODUCTION

Melanocytes are the pigment-producing cells primarily found at the epidermal-dermal junction. Melanoma is an aggressive form of skin cancer originating from melanocytes. Environmental factors, particularly ultraviolet radiation (UVR) exposure, and various phenotypic risk factors link to the pathogenesis of melanoma.

Cancer stem cells (CSCs) within melanoma play a crucial role in tumor progression, therapeutic resistance, and recurrence due to their capabilities for self-renewal, differentiation, and immune evasion. One of the critical pathways implicated in the regulation of these CSCs is the Sonic Hedgehog (SHH) signaling pathway. The SHH signaling pathway is essential for the regulation of cell differentiation, proliferation, and tissue polarity. It is well-known for its role in embryonic development and its involvement in various cancers, including basal cell carcinoma and medulloblastoma. In the context of melanoma, the SHH pathway significantly influences CSC behavior, promoting tumorigenicity, metastasis, and resistance to conventional therapies. This pathway operates through canonical mechanisms involving the activation of Gli transcription factors, as well as non-canonical mechanisms that contribute to cell migration and cytoskeletal organization. Targeting the SHH pathway has emerged as a promising therapeutic strategy for treating melanoma. Preclinical and clinical settings have explored various inhibitors targeting components of the SHH pathway, such as Smoothened (Smo) and Gli proteins. However, the development of resistance to these inhibitors necessitates the ongoing investigation of new therapeutic combinations and novel inhibitors to improve patient outcomes. Current research is focused on combining SHH pathway inhibitors with other therapies, including immunotherapies, to achieve more effective and durable responses. The understanding of SHH signaling in CSCs underscores the complexity of melanoma biology and the need for innovative treatment strategies. By disrupting the SHH signaling mechanisms that maintain CSC properties and contribute to tumor progression, targeted therapies aim to eliminate the root causes of tumorigenesis and improve long-term survival rates for melanoma patients.

Overview of Malignant Melanoma

Malignant melanoma stems from the pigment-producing cells called melanocytes. These cells originally derived from the neural crest cells and migrate to the basal layer of the epidermis of various parts of the body, including the skin, hair, uveal tract, meninges, and mucosa (1). These melanocytes are primarily located at the epidermal-dermal junction of the skin, which is where the majority of cutaneous melanomas develop (2). Malignant melanoma is one of the top five or six most common cancers and has the highest mortality rate among skin cancers. It accounts for 5% of cancer cases in men and 4% in women (3) and contributes to 75% of all skin cancer deaths (2,4,5). The formation of malignant melanoma is primarily driven by unrepaired DNA damage in skin cells, most commonly caused by ultraviolet radiation (UVR) from sun exposure or tanning beds (6). This DNA damage leads to genetic mutations that trigger rapid multiplication of the affected skin cells, resulting in the formation of malignant tumors (7). These tumors consist of heterogeneous populations of tumor cells, including CSC which possess highly tumorigenic and chemoresistant properties (8–10).

Cancer Stem Cells in Melanoma

CSCs play a pivotal role in the progression and treatment resistance of malignant melanoma. Identified through specific biomarkers such as ABCB5, NGFR (CD271), and ALDH, CSCs are instrumental in melanoma initiation and progression due to their prolonged self-renewal capacity, vasculogenic differentiation, and

immune evasion abilities (11,12). These CSCs contribute significantly to tumor heterogeneity. These cells are characterized by their highly tumorigenic and chemo resistant properties. They facilitate metastatic dissemination and therapeutic resistance, leading to malignant recurrence (13,14). The features of CSCs, such as low immunogenic profiles and the ability to escape immune detection, allow them to survive standard treatments and contribute to tumor relapse. Consequently, targeting this subpopulation is considered an attractive strategy to improve therapeutic outcomes (14). Recent research has provided pre-clinical proof-of-concept for the potential therapeutic utility of targeting CSCs in melanoma. Strategies that combine CSC-targeting agents with standard drugs may offer enhanced therapeutic options for melanoma patients (14). These approaches weight the unique properties of CSCs, such as quiescence and the expression of certain enzymes like ALDH, to overcome therapy resistance and prevent recurrence (13). CSCs in melanoma are also associated with unique metabolic reprogramming, which supports their survival and function within the tumor microenvironment. Advances in understanding the cellular and molecular biology of these cells have revealed their involvement in critical processes such as tumor dissemination, epithelial-to-mesenchymal transition (EMT), and angiogenesis, mediated by specific intracellular signaling pathways (14,15). Given the aggressive nature of malignant melanoma and its high drug resistance, innovative therapeutic strategies targeting CSCs hold promise. By disrupting the regulatory networks that maintain CSC characteristics and contribute to tumor metastasis, these therapies aim to eradicate the root causes of tumor progression and resistance. This will ultimately allow improving patient outcomes (16,17).

SHH Signalling in Melanoma

The SHH signaling pathway has been extensively studied in different cancer types, both because it is an important signaling pathway of developmental biology and because developmental processes are like cancer. In addition to chemical agents that inhibit the SHH pathway, such as cyclopamine, direct silencing of molecules in this pathway has been used to identify potential molecules and therapeutic targets. Cell culture-based melanoma studies have shown that transient silencing of Smo and Gli1 in normoxia and hypoxia decreases the invasion capacity of cells (18). Another study reported that Gli1 gene expression is an important marker for melanoma and other cancer types. Silencing of Gli1 gene expression in an in vivo melanoma model suppresses the SHH pathway and is associated with the Kras/Akt signaling pathway (19). Kras/Mek/Erk signalling in SHH pathway regulates the localization and translocation of Gli1 to the nucleus (19). The mutations in Gli1 gene have been reported as a biomarker for melanoma susceptibility in melanoma patients (20).

Due to the Braf mutation in melanoma, Braf based studies are critical. PDGFR alpha, which is one of the molecules induced by SHH leads to the melanoma Braf insensitivity. With PDGFR alpha and Smo antagonist LDE225i increase the Braf sensitivity in melanoma (21).

SHH Signaling Pathways in Cancer Stem Cells

The SHH signaling pathway is fundamental to how cells grow, differentiate, and establish their organization within tissues. It plays an indispensable role in shaping the central nervous system (CNS) during vertebrate development, guiding the formation of complex patterns, determining cell fate, and directing axon growth (22). This pathway ensures not only the proliferation and survival of neural cells but also their specialization into different types of neurons. The key players in SHH signaling are the Gli transcription factors, Gli1, Gli2, and Gli3. Each of these molecules contributes differently to the pathway's functions (23). SHH signaling operates through two distinct mechanisms as the canonical and non-canonical pathways. Both mechanisms crucial but with specific and diverse roles in cellular regulation (22,23). Once SHH pathway is activated, Gli1 translocates to the nucleus. It drives the transcription of specific target genes. The activation of these genes is critical for various cellular processes. This pathway is not only essential for maintaining normal embryonic and somatic stem cell function but is also highly active in CSCs, contributing to their unique characteristics of self-renewal and tumor initiation. Gli1 activation in CSCs has been extensively studied and found to significantly upregulate several key regulatory molecules, including CD133, Hif1a, histone deacetylases (HDACs), and matrix metalloproteinases (MMPs).

These molecules play pivotal roles in maintaining CSC properties such as tumorigenicity, resistance to therapy, and metastatic potential, making the SHH-Gli1 axis a critical target for therapeutic intervention in cancer treatment. (Figure-1) (24–29).

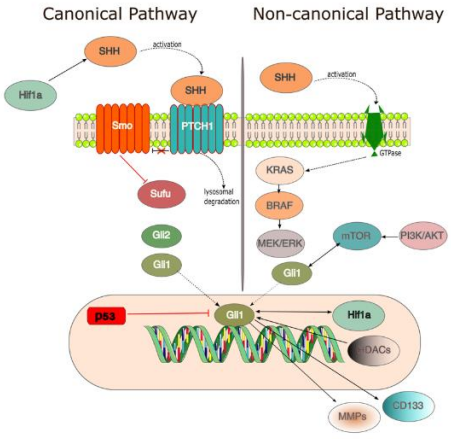

Figure-1. Canonical and non-canonical SHH pathways. Both types of pathways lead to the activation of the Gli1 protein, which translocates into the nucleus to drive target gene expression. This pathway is active in embryonic and somatic stem cells, as well as cancer stem cells. Gli1 activation has been shown to upregulate key molecules in cancer stem cell biology, including CD133, Hif1a, HDACs, and MMPs. The schematic diagram was created by Berrin Ozdil, using Inkscape, an open-source software.

In canonical SHH signaling, the pathway gets triggered when the SHH glycoprotein binds to the receptor Patched (Ptch1), which spans the cell membrane. Normally, without SHH, Ptch1 suppresses the activity of another membrane protein called Smo. However, when SHH attaches to Ptch1, this suppression is lifted, allowing Smo to accumulate and activate the pathway's downstream components, primarily the Gli proteins. The movement of Smo into the cell membrane is essential for activating the Gli proteins. Once activated, these proteins move to the cell nucleus, where they initiate the transcription of target genes like Ptch1 and Gli1. These molecules create both negative and positive feedback loops to regulate the pathway (23). Among the Gli proteins, Gli1 acts as a full-length transcriptional activator. In contrast, Gli2 and Gli3 can either activate or suppress gene expression depending on how they are processed after transcription. In the absence of SHH signaling, Gli2 and Gli3 are converted into their repressor forms, known as Gli2R and Gli3R, which help maintain a balance between turning the pathway on and off.

The non-canonical SHH signaling pathway operates independently of the primary Gli transcriptional activity. This involves other molecules such as G-protein-coupled receptors (GPCRs) and small GTPases like RhoA. Emerging evidence suggests that the non-canonical pathway plays a role in processes such as cytoskeletal organization and cell migration (23). The selection mechanism between canonical and non-canonical routes remains a subject of investigation, indicating the complexity and versatility of the SHH pathway (23).

Aberrant activation of the SHH signaling pathway has been implicated in a variety of cancers, including basal cell carcinoma, medulloblastoma, and gliomas (13,30). The dysregulation of SHH signaling can promote tumorigenesis, tumor progression, and therapeutic resistance (30). One of the most significant components of this pathway in cancer is Smo. Smo mediates the transfer of transcription factors. Targeting Smo has become a primary strategy in cancer therapeutics, particularly for basal cell carcinoma and medulloblastoma, where Smo inhibitors have shown greater effectiveness (23).

CSCs are known for their capacity for self-renewal, differentiation, and tumorigenicity, which contribute to cancer initiation, progression, and resistance to therapy (11,12). These unique properties are maintained through the activation of several signaling pathways, including SHH, Wnt, and Notch, which are also essential for cancer and stem cell function (23) (Table 1). The

SHH signaling pathway is pivotal in regulating CSC behavior, particularly in melanoma. This pathway operates through the SHH ligand binding to its receptor Ptch, which subsequently activates Smo protein. Activated Smo leads to the translocation and activation of Gli transcription factors, which promote the expression of genes responsible for cell proliferation, survival, and stemness (31). SHH is particularly significant in coordinating CSC-driven processes such as tumor initiation, maintenance, and metastasis.

Similarly, the Wnt and Notch signaling pathways are crucial for CSC function. The Wnt pathway is involved in regulating cell fate determination, polarity, and migration (23). Aberrant Wnt signaling has been shown to promote tumorigenesis by sustaining CSC stemness and enhancing their metastatic potential. Wnt signaling not only supports CSC self-renewal but also contributes to resistance against therapies, particularly through β-catenin-mediated transcriptional activation of survival genes (32,33). The Notch pathway, on the other hand, plays a critical role in cell-cell communication and tumor microenvironment interactions. Notch signaling regulates CSC differentiation and quiescence, enabling these cells to evade immune surveillance and persist under therapeutic pressure. Dysregulation of Notch signaling has been associated with increased CSC-mediated tumor heterogeneity and adaptability (34). Interestingly, these pathways are not mutually exclusive, they often interact and converge on common downstream targets, amplifying their effects on CSC behavior. For instance, SHH and Wnt signaling are known to cooperate in maintaining the stem cell niche, while Notch signaling modulates the activity of both pathways to fine-tune CSC differentiation and proliferation (35,36). In melanoma CSCs, the activation of SHH signaling has been linked to enhanced tumorigenicity and resistance to conventional therapies, such as chemotherapy and radiotherapy (17). CD133, a well-established cancer stem cell marker, has been associated with hypoxia-inducible factor 1-alpha (Hif1a), which is upregulated in tumors under hypoxic conditions. In response to hypoxia, elevated levels of Hif1a enhance the activation of the SHH signaling pathway via Gli transcription factors, leading to an increased expression of CD133 within the cell (37). This phenomenon has been demonstrated in hepatocellular carcinoma through SHH/Gli activation in cancer cells via SCUBE1 secreted by cancer-associated fibroblasts (38). The SHH signaling pathway plays a critical role in the regulation of CSCs, including those specifically identified in melanoma, as supported by our recent studies (29). Melanoma CSCs display higher expression levels of Gli1 and Ptch2 genes compared to non-stem cancer cells. Additionally, silencing SHH significantly reduces Gli1 and Ptch2 expression in CD133+ melanoma cells. Similarly, silencing Hif1a also leads to a decrease in both Gli1 and Ptch2 expression (29). Melanoma CSCs exhibit unique metabolic reprogramming and low immunogenic profiles, enabling them to evade the immune system. This is facilitated by the SHH pathway, which promotes the secretion of immunosuppressive factors and negative modulation of T cell functions, thus allowing CSCs to escape immune surveillance (16). Additionally, SHH signaling is implicated in processes such as epithelial-to-mesenchymal transition (EMT) and angiogenesis, which are critical for tumor dissemination and metastasis (13).

Table-1. Comparative Analysis of SHH, Notch, and Wnt Pathways in Cancer Biology

Feature	SHH Pathway	Wnt Pathway	Notch Pathway
Primary Function	Cell fate determination, stemness regulation	Cell differentiation, maintenance of stem cells.	Cell proliferation, polarity, and migration.
Key Components	SHH ligand, PTCH1, SMO, GLI transcription factors.	Notch receptors (Notch1-4), Delta/Jagged ligands.	Wnt ligands, Frizzled receptors, β-catenin.
Role in Cancer	Promotes tumor growth, stemness, and metastasis.	Enhances angiogenesis, drug resistance, and EMT.	Regulates proliferation, metastasis, and tumor microenvironment.
Pathway Crosstalk	Interacts with Wnt and Notch pathways.	Crosstalk with SHH and Wnt in stem cell niches.	Modulates SHH and Notch in developmental processes.

Therapeutic Implications

Targeting SHH pathway presents a promising strategy for eradicating CSCs in melanoma. However, single-pathway inhibitors often face challenges such as adaptive resistance and incomplete tumor eradication. Combining SHH pathway inhibitors with specific pathways (Wnt, Notch etc.) may enhance therapeutic efficacy by disrupting multiple facets of CSC regulation. Current research is exploring such combinatorial approaches, highlighting the potential for more durable clinical responses in melanoma treatment.

Melanoma has seen significant advancements in the realm of targeted therapies, specifically those aimed at inhibiting the Hedgehog (Hh) signaling pathway, which includes Smo and Gli inhibitors (23,39). The primary target for these inhibitors in clinical trials has been Smo. However, resistance mechanisms to Smo inhibitors have been identified, necessitating the exploration of new Hh pathway inhibitors to effectively control tumorigenesis (23). For example, the U.S. Food and Drug Administration (FDA) approved GDC-0449 (Vismodegib) in 2012 as a standard therapy for patients with locally advanced and metastatic basal cell carcinoma (BCC). Subsequent studies focused on combining GDC-0449 with temozolomide (TMZ) to evaluate its efficacy in both pediatric and adult patients with recurrent or refractory medulloblastoma (MB) (23).

The complexity of melanoma and its tendency to develop resistance to treatments have led researchers to explore combination therapies. Targeted therapies, while effective at first, often lead to quick but short-lived responses because of resistance. On the other hand, immunotherapies, like immune checkpoint inhibitors, tend to produce longer-lasting effects but can take time to work (40). By combining targeted therapies with immunotherapies, there's the potential to achieve both a fast and strong anti-tumor response along with long-term benefits for patients. Currently, clinical trials are investigating various combinations, such as dabrafenib (with or without trametinib) alongside ipilimumab for patients with Braf V600E/K-mutated metastatic melanoma. These trials aim to determine the best approach in terms of managing side effects, timing, and the sequence of administering these therapies. The goal is to develop strategies that can overcome treatment resistance and provide lasting responses for melanoma patients(40).

The discovery of molecularly effective targeted therapies, particularly Hh inhibitors, has markedly improved the treatment landscape of melanoma. The focus has been on Smo and Gli inhibitors, with clinical trials validating their therapeutic potential in preclinical SHH-MB models (39). Despite the clinical use of Smo antagonists not being fully established, future Gli inhibitors and multitargeting approaches appear promising for MB patients (39). Evidence-based recommendations on the pharmacokinetics and pharmacodynamics of Hh inhibitors like sonidegib and vismodegib highlight their therapeutic considerations in special patient populations (41). Differences in their pharmacokinetic profiles may influence their efficacy and safety, suggesting a need for direct comparative clinical trials to determine their clinical relevance (41).

CONCLUSION

Malignant melanoma presents a significant challenge in skin cancer treatment due to its aggressive behavior and resistance to therapies. The critical role of cancer stem cells (CSCs) in driving melanoma progression, therapy resistance, and recurrence underscores the urgent need for innovative treatment strategies. A significant factor in this resistance is the presence of CSCs, which can renew themselves, differentiate into various cell types, and evade the immune system. The SHH pathway, with its dual roles in canonical and non-canonical signaling, emerges as a critical regulator of CSC properties, influencing tumorigenicity, metastasis, and resistance to conventional therapies. Targeting the SHH pathway has shown promise in preclinical and clinical settings. Besides, it is crucial to explore how SHH interacts with other pathways such as Wnt and Notch, which also regulate stemness, differentiation, and survival. The emergence of resistance to SHH inhibitors requires continuous exploration of novel therapeutic combinations and agents. A promising hypothesis is the integration of SHH pathway inhibitors with immunotherapies and targeted therapies that disrupt crosstalk with other molecules. This approach could reveal synergistic effects, overcoming resistance mechanisms and leading to more effective, durable responses in melanoma treatment. As the field advances, a deep understanding of the SHH signaling mechanisms and the development

of next-generation inhibitors hold the potential to disrupt the regulatory networks sustaining CSCs. By focusing on these innovative approaches, the goal is to eliminate the main causes of tumorigenesis and significantly improve long-term survival rates for patients with melanoma. Continuous efforts in this research area are vital for developing innovative treatments that can overcome the current challenges and provide better outcomes for melanoma patients.

Funding

This research was funded by the Scientific Research Projects Coordination Unit at Ege University under Project Number 20785.

Acknowledgement

The authors thank the Scientific Research Projects Coordination Unit at Ege University for their financial support.

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