ATYPICAL NEVI AND MELANOMA: PHENOTYPIC AND MOLECULAR PERSPECTIVES

CRISTINA-RALUCA MIHULECEA*, MARIA ROTARU**

Summary

Melanoma is among the most aggressive skin cancers, making early detection essential for optimizing patient prognosis. In current clinical practice, clinical examination, dermoscopy, and histopathology represent the standard methods for assessing melanocytic lesions; however, modern molecular techniques, such as fluorescence in situ hybridization (FISH), can provide additional information in cases with ambiguous features.

This research focused on four main directions: analyzing clinical and dermoscopic similarities between atypical nevi and early-stage melanoma, examining the role of the tumor microenvironment in melanoma, assessing the diagnostic utility of FISH, and synthesizing the current knowledge on nevogenesis and melanomagenesis. By integrating clinical, histopathological, and molecular data, the study highlighted both the phenotypic overlap between benign and malignant lesions and the molecular features that can aid diagnosis in challenging cases.

FISH techniques can complement histopathological evaluation in lesions with ambiguous architecture by highlighting relevant genetic alterations. The analysis of the tumor microenvironment confirms its involvement in melanocytic progression, while the synthesis of current literature enhances the understanding of mechanisms involved in melanoma development.

This article summarizes the main results of the doctoral thesis entitled "Phenotypic and Molecular Aspects in Atypical Nevi and Melanoma," which aimed to provide an integrated analysis of melanocytic lesions through clinical, dermoscopic, histopathological, and molecular assessment. Overall, the study underscores the importance of a multidimensional approach in the diagnosis and management of melanocytic lesions and highlights the value of modern molecular techniques in clarifying diagnostically difficult cases.

Keywords: *atypical nevi, melanoma, FISH technique, diagnosis, treatment, molecular.*

Received: 4.12.2025 *Accepted: 20.01.2026*

Introduction

Melanoma is an aggressive neoplasm with unpredictable evolution, and the early identification of suspicious lesions is essential for improving patient prognosis. As one of the tumors with the highest number of somatic mutations [1], melanoma can sometimes be difficult to differentiate from atypical nevi, especially in early stages, due to its marked morphological heterogeneity. These diagnostic challenges highlight the importance of a comprehensive evaluation that integrates clinical, dermoscopic, histopathological, and molecular information.

The role of the tumor microenvironment, the interactions between stromal and melanocytic cells, and the involvement of angiogenic processes are essential factors both in nevus formation and in melanoma progression [2]. Current research shows that cancer-associated fibroblasts, inflammatory cells, mast cells, and the microvascular network can influence the

* Doctoral Studies-Department II – Microscopic Morphology/Histology, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania.

** Prof. Univ. Dr. Maria Rotaru Medical Center – DermaTop Medical Clinic, Sibiu, Romania.

dynamics of melanocytic transformation [2]. Thus, the analysis of tumor microenvironment components becomes a valuable tool for understanding the onset and evolution of melanoma [2].

Beyond phenotypic assessment, modern molecular techniques provide additional objectivity in lesions with unclear morphology. Fluorescence in situ hybridization (FISH) has emerged as a complementary method in the differential diagnosis of melanocytic lesions, by detecting genetic abnormalities relevant for lesion classification [3]. Correlating FISH results with clinical, dermoscopic, and histopathological data contributes to a more accurate characterization of lesions and supports the confirmation or exclusion of malignancy.

In this context, the present study integrated the analysis of phenotypic overlaps between atypical nevi and melanoma, the interactions between the tumor microenvironment and melanoma, the evaluation of standard and modern diagnostic techniques, as well as a synthesis of the current data on the mechanisms involved in nevogenesis and melanomagenesis. This approach encompassed clinical and histological evaluation, along with an analysis of the etiological pathways and modes of melanoma onset – whether arising de novo or on pre-existing nevi. By bringing together clinical, -dermoscopic, histopathological, and molecular perspectives, the study aims to provide an integrated view of melanocytic lesions, emphasizing the complexity of differential diagnosis and the need for modern techniques in clarifying diagnostically challenging cases.

Material and Methods

The research methodology of the doctoral thesis titled "Phenotypic and Molecular Aspects in Atypical Nevi and Melanoma" was built on an integrated approach combining clinical, dermoscopic, histopathological, and molecular analysis of the melanocytic lesions examined.

An extensive review of the relevant literature was first conducted to establish the theoretical foundation of the study, formulate the working hypotheses, and define the main research directions. Subsequently, representative melanocytic lesions – common nevi, atypical nevi, and melanomas – were selected and evaluated clinically and dermoscopically using standardized criteria for the assessment of phenotypic characteristics. Histopathological examination complemented these findings by analyzing tissue architecture, the degree of differentiation, and other features relevant to the diagnosis of melanocytic tumors.

The study also included a comparative analysis of the clinical and dermoscopic features of atypical nevi versus melanoma, using diagnostic algorithms such as the ABCDE rule, "Chaos & Clues" [4], Pattern Analysis, and the 7-point checklist, to highlight phenotypic overlaps between these lesions. Additionally, the research explored the multiple roles of dermoscopy, including its contribution to the estimation of tumor thickness.

An important component of the methodology involved the assessment of the tumor microenvironment, including the analysis of vascularization, mast cell density, and peritumoral stromal interactions, to understand their contribution to the initiation and progression of melanocytic transformation.

The molecular dimension of the research was carried out using the FISH technique on paraffin-embedded tissue sections, employing the Vysis Melanoma FISH kit (Abbott Molecular), which includes probes targeting RREB1 (6p25), MYB (6q23), CCND1 (11q13), and CEP6. The analysis was performed in collaboration with a pathologist, and the results were interpreted according to internationally validated criteria, then correlated with clinical, dermoscopic, and histopathological results.

In parallel, a theoretical synthesis was conducted regarding the mechanisms of nevogenesis and melanomagenesis, as well as the patterns through which melanoma may arise – either from pre-existing nevi or de novo – providing a conceptual framework for interpreting the study results. All data were compiled into a database and statistically analyzed, contributing to an integrated evaluation of the phenotypic and molecular characteristics of melanocytic lesions.

Results

The analysis of the examined material revealed a series of phenotypic, histopathological, and molecular features relevant for distinguishing melanocytic lesions and for understanding the mechanisms involved in their progression.

At the phenotypic level, clinical and dermoscopic analysis of melanocytic lesions showed significant overlaps between atypical nevi and early melanoma, particularly regarding asymmetry, color variability, and similar dermoscopic structures [5]. However, systematic assessment of dermoscopic criteria allowed the identification of features suggestive of melanoma, such as polymorphous vessels, blue-gray structures, shiny white lines, eccentric structureless areas, or irregular pigment patterns [5], [6]. Histopathological examination complemented the dermoscopic evaluation, revealing architectural and cytological alterations characteristic of malignant lesions – including junctional irregularity, marked melanocytic atypia, focal regression, and stromal remodeling accompanied by variable inflammatory infiltrates [5], [6]. Comparing these features with those found in common and atypical nevi enabled the identification of diagnostic patterns useful for recognizing suspicious lesions [5], [6]. The use of standardized dermoscopic algorithms proved essential in correctly stratifying melanocytic lesions, particularly when clinical and dermoscopic criteria were ambiguous [5], [6].

The assessment of tumor thickness based on dermoscopic colors and structures showed only limited concordance with the histopathological Breslow index. Although certain dermoscopic clues – such as blue pigmentation or regression structures – suggested deeper invasion, they did not consistently correlate with the true tumor depth [6]. One melanoma, for example, showed intense blue coloration dermoscopically but had a superficial thickness on histopathological examination [6]. Overall, dermoscopy was useful for identifying lesions requiring excision, but it was not sufficiently precise for estimating tumor thickness [6].

The evaluation of the tumor micro-environment highlighted the complex role of stromal cells and the vascular network in the dynamics of melanocytic progression [2]. It was demonstrated that, as lesions evolve from nevi to melanoma, progressive changes occur within the stromal architecture, including extracellular matrix remodeling, increased density of activated fibroblasts, and intensified inflammatory infiltrates [2]. A central element of these transformations is the interaction between mast cells and vascular components. Through their ability to release proangiogenic and immunomodulatory mediators, mast cells may influence the formation of new blood and lymphatic vessels, thereby supporting tumor expansion and invasion. Additionally, the evaluation of lymphatic vessels revealed increased density in areas adjacent to tumors, an essential step in melanoma dissemination. Overall, the results indicate that the tumor microenvironment is not a passive structure, but an active participant in melanoma biology, influencing both lesion initiation and tumor progression [2].

The application of FISH provided additional molecular information, complementing morphological findings and revealing genetic abnormalities suggestive of malignancy in lesions with inconclusive features [3]. Correlating FISH results with clinical and histopathological data demonstrated its usefulness in clarifying diagnoses and confirming melanoma in lesions with atypical architecture [3]. The findings showed that FISH can be particularly valuable in confirming the benign nature of certain atypical nevi [3]. In melanomas, the genetic profiles observed were heterogeneous, reflecting the biological diversity of the lesions included in the study [3]. This variability suggests that the usefulness of FISH in routine practice depends on the specific characteristics of each lesion, supporting its role as a complementary tool in cases with ambiguous morphology [3] (see *Figure 1 – FISH probes in melanoma*). The chromosomal abnormalities identified involved regions known to play a role in melanomagenesis, but their uneven distribution among cases highlights differences in FISH responsiveness depending on lesion subtype and architecture [3].

Analysis of data regarding melanoma development mechanisms revealed the complexity of melanocytic transformation and the distinct

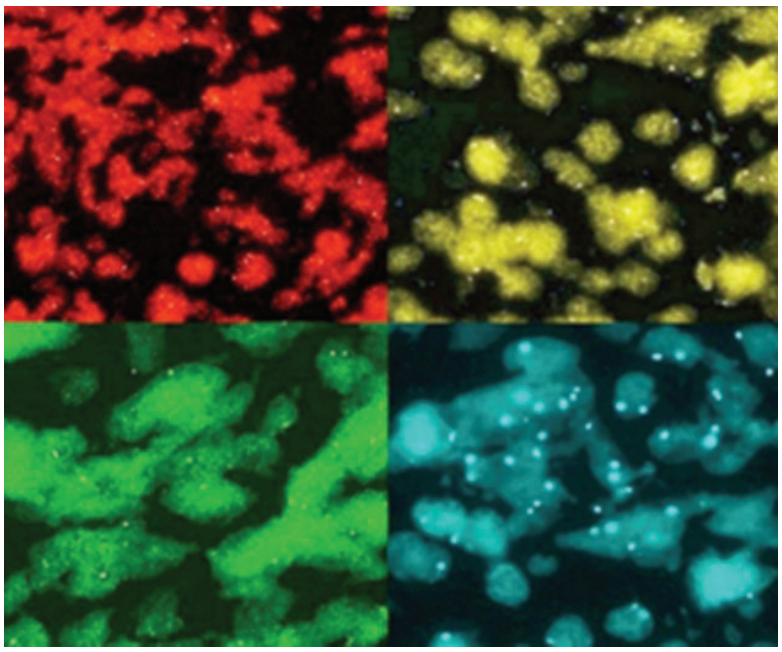

Figure 1. FISH probes in melanoma. Legend: RREB1 (6p25) – red; MYB (6q23) – yellow (gold); CCND1 (11q13) – green; CEP6 (centromere 6) – aqua. This figure was previously published and is reproduced with permission from Spandidos Publications. Adapted from: Mihulecea CJ, Ceaușu AR, Gaje NP, Rotaru M, Raica M. Fluorescence in situ hybridization in melanoma diagnosis: Pros and cons. Experimental and Therapeutic Medicine. 2025;29(6):118. doi:10.3892/etm.2025.12868.

roles of genetic, phenotypic, and environmental factors in disease initiation and progression [7]. The literature indicates that melanoma may arise either on pre-existing nevi or de novo, with the two mechanisms displaying different biological and phenotypic characteristics [7]. The results suggest that linear progression from nevus to melanoma is possible, but not the predominant pathway. Many melanomas arise independently, supporting the existence of distinct biological routes of melanomagenesis [7]. Furthermore, factors such as ultraviolet radiation, genetic predisposition, and molecular instability appear to act synergistically, contributing to the variability in clinical and histological presentation [7]. Integrating these data offers a clearer understanding of nevogenesis and melanomagenesis and underscores their importance in optimizing prevention, diagnostic, and monitoring strategies [7].

Overall, the findings support the idea that both the tumor microenvironment and the genetic profile play major roles in differentiating atypical nevi from melanoma, and that combining standard and modern diagnostic methods can improve diagnostic accuracy in challenging lesions with atypical morphology.

Discussions

The analysis of the data obtained in the doctoral thesis titled "Phenotypic and Molecular Aspects in Atypical Nevi and Melanoma" demonstrated that melanocytic lesions form a biological continuum in which phenotypic overlap, histological variability, and molecular heterogeneity may generate significant challenges in the differential diagnosis between atypical nevi and melanoma.

Phenotypically, clinical and dermoscopic evaluation revealed notable overlaps between atypical nevi and early melanoma, particularly regarding asymmetry, color variegation, and certain shared dermoscopic structures [5], [6]. Nevertheless, the systematic use of standardized dermoscopic algorithms enabled the identification of features suggestive of malignancy – such as polymorphous vessels, blue-gray structures, shiny white lines, eccentric structureless

areas, and irregular hyperpigmented areas – which, when interpreted collectively and correlated with clinical presentation, contributed to improved diagnostic accuracy [5], [6]. A study by Lallas et al. demonstrated that irregular hyperpigmented areas are markers suggestive of melanoma compared to atypical nevi [8], a finding confirmed in the present study. Histopathological examination further refined the evaluation by highlighting architectural and cytological features characteristic of malignant lesions, including junctional disarray, marked melanocytic atypia, focal regression, and stromal remodeling with variable inflammatory infiltrates [5], [6]. Comparison with features observed in common or atypical nevi allowed the delineation of diagnostic patterns useful for identifying ambiguous lesions [5], [6].

The assessment of tumor thickness based on dermoscopic colors and structures showed variable reliability. Although dermoscopy may reveal clues suggesting deeper invasion – such as blue pigmentation, regression structures – these did not consistently correlate with the histopathological Breslow index [6]. This discrepancy aligns with recent literature [9], which indicates that dermoscopy may help distinguish thin from invasive melanomas [10], but cannot replace histopathology for precise measurement.

The assessment of the tumor microenvironment showed that the stroma plays an active role in the dynamics of melanocytic progression [2]. Extracellular matrix remodeling, increased density of activated fibroblasts, and intensified inflammatory infiltrates observed in malignant lesions are consistent with recent literature describing the tumor microenvironment as an essential participant in the initiation and progression of melanoma [2]. Mast cells exhibited an increased distribution in both tumor and peritumoral areas, and their correlation with microvascular density supports their involvement in angiogenic and lymphangiogenic processes. Similarly, a study by Bahri et al. reported the presence of mast cells both peri- and intratumorally [11]. The potential role of these cells as therapeutic targets in preventing tumor progression and metastasis has also been discussed [12]. By releasing pro-angiogenic and immunomodulatory mediators, mast cells may facilitate tumor invasion and dissemination. The evaluation of lymphatic vessels revealed increased density in areas adjacent to tumors, a finding consistent with the hypothesis that lymphangiogenesis represents an important step in melanoma metastasis. Taken together, these findings confirm that the tumor microenvironment is not a passive structure but an active contributor, influencing both melanocytic transformation and tumor progression [2].

The application of FISH provided additional molecular information, complementing the clinical, dermoscopic, and histopathological evaluation in lesions with ambiguous architecture or phenotype. The use of RREB1, MYB, CCND1, and CEP6 probes allowed the investigation of genomic regions associated with melanomagenesis, revealing a generally stable genetic profile in atypical nevi, supporting their benign nature [3]. This concordance highlights the value of FISH as a method for excluding malignancy in lesions with unusual clinical features or phenotypic overlap with melanoma [3]. In melanomas, the identified abnormalities showed considerable variability, reflecting the biological heterogeneity of these tumors [3]. Some lesions demonstrated alterations in the investigated regions, whereas others did not meet the molecular criteria of the panel – an observation widely reported in the literature [3]. This confirms that FISH performance depends on the characteristics of each tumor and that the method is most useful as a complementary tool within a multimodal diagnostic approach. Consistent with our findings, other studies have shown that FISH accuracy improves when results are interpreted in conjunction with clinical and histopathological data, reinforcing its role as an adjunct rather than a standalone diagnostic technique [13].

The analysis of mechanisms involved in nevogenesis and melanomagenesis demonstrated that melanoma may arise either on preexisting nevi or de novo, the two pathways exhibiting distinct biological characteristics [7]. Linear progression from nevus to melanoma, although possible, does not appear to be the predominant mechanism, as most melanomas develop de novo [7]. The interaction between ultraviolet exposure, genetic predisposition, and

molecular instability contributes to the variability observed in clinical and histological presentations, highlighting the complexity of melanocytic transformation [7]. Further research is needed to clarify the molecular mechanisms of melanoma and their clinical implications. Melanoma development is influenced by multiple factors, including UV radiation, genetic susceptibility, skin phototype, immunosuppression, viral infections, and nevus count [7].

Overall, the results support the value of integrating clinical, dermoscopic, histopathological, and molecular evaluation for a more precise characterization of melanocytic lesions. This multimodal approach improves the assessment of atypical lesions and contributes to more accurate diagnosis and management, particularly in morphologically challenging cases.

Conclusions

The study highlights the complexity of melanocytic lesions and underscores the importance of a multidimensional evaluation in their understanding and characterization. The phenotypic overlap between atypical nevi and early melanoma, together with the observed histological and molecular variability, emphasizes the need for careful correlation of clinical, dermoscopic, histopathological, and molecular data to achieve an accurate interpretation.

The analysis of the tumor microenvironment revealed the active role of mast cells, as well as of the blood and lymphatic vessels, in the initiation and progression of melanoma. Interactions among these components support angiogenic and lymphangiogenic processes, reinforcing the literature that identifies the tumor microenvironment as a key factor in invasion and dissemination.

Regarding nevogenesis and melanomagenesis, the study demonstrates that melanoma may arise both from pre-existing nevi and de novo, each pathway presenting distinct biological characteristics. The diversity of these mechanisms – shaped by genetic, phenotypic, and environmental factors – highlights the multiple routes through which melanoma can develop and the importance of continuous dermoscopic monitoring.

The FISH technique provided valuable additional molecular information, particularly in lesions with ambiguous architecture or phenotype. Although the panel used has certain limitations, its ability to confirm the benign nature of atypical nevi and to reveal chromosomal abnormalities in suspicious lesions strengthens the role of molecular investigations in contemporary dermatologic diagnostics.

Overall, the conclusions support the adoption of an integrated approach in the evaluation of melanocytic lesions, enhancing diagnostic precision and guiding therapeutic decisions. The practical relevance of the study lies in demonstrating the impact of combining traditional and modern methods on understanding the onset – an essential aspect for prevention, surveillance, and early diagnosis.

Acknowledgements

Acknowledgements are addressed to the academic staff, medical personnel, and institutions that supported the completion of this research by providing scientific and professional assistance.

Warm thanks are extended to Professor Maria Rotaru for selecting the research topic "Phenotypic and molecular aspects in atypical nevi and melanoma" and for providing access to the clinical and dermoscopic documentation from the DermaTop Clinic.

This research was funded through a doctoral grant awarded in 2019, within the "Victor Babeș" University of Medicine and Pharmacy of Timișoara (UMFVBT) (project acronym: AFMNAM), under the supervision of Prof. Univ. Dr. Maria Rotaru. We would like to thank Prof. Univ. Dr. Marius Raica and the team of the Department II – Microscopic Morphology/Histology (UMFVBT) for the guidance and support provided throughout the doctoral research period.

Bibliography

1. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature [Internet].2013;500(7463):415–21.
2. (Jitian) Mihulecea CR, Ceaușu A, Gaje N, Rotaru M, Raica M. Review: The tumor microenvironment of melanoma. Medicine in Evolution. 2024 Sep 30;30(3):414–21.
3. (Jitian) Mihulecea CR, Ceaușu A, Nela Gaje, Rotaru M, Raica M. Fluorescence in situ hybridization in melanoma diagnosis: Pros and cons. Experimental and Therapeutic Medicine. 2025 Apr 14;29(6):1–13.
4. Rosendahl C, Cameron A, McColl I, Wilkinson D. Dermatoscopy in routine practice - 'chaos and clues'. Aust Fam Physician. 2012 Jul;41(7):482-7.
5. Jitian (Mihulecea) CR, Frățilă S, Rotaru M. Clinical-dermoscopic similarities between atypical nevi and early stage melanoma. Experimental and Therapeutic Medicine. 2021 Jun 9;22(2).
6. (Jitian) Mihulecea CR, Iancu GM, Leventer M, Rotaru M. (2023). The Many Roles of Dermoscopy in Melanoma Detection. Life, 13(2), 477.
7. (Jitian) Mihulecea CR, Rotaru M. Review: The Key Factors to Melanomagenesis. Life. 2023 Jan 8;13(1):181.
8. Lallas A, Longo C, Manfredini M, Benati E, Babino G, Chinazzo C, et al. Accuracy of Dermoscopic Criteria for the Diagnosis of Melanoma In Situ. JAMA Dermatology. 2018 Apr 1;154(4):414.
9. Martínez-Piva MM, Vacas AS, Rodríguez Kowalczuk MV, Gallo F, Rodrígues Vasconcelos M, Mazzuoccolo LD. La dermatoscopia como herramienta para inferir el Breslow del melanoma. Actas Dermo-Sifiliográficas. 2021 May 1;112(5):434–40.
10. Rodríguez-Lomba E, García-Piqueras P, Lozano-Masdemont B, Nieto-Benito LM, Hernández de la Torre E, Parra-Blanco V, Suárez-Fernández R, Lázaro-Ochaita P, Avilés-Izquierdo JA. 'Rainbow pattern': a dermoscopic sign of invasive melanoma. Clin Exp Dermatol. 2022 Mar;47(3):529-533.
11. Bahri R, Kiss O, Prise I, Garcia-Rodriguez KM, Haris Atmoko, Martínez-Gómez JM, et al. Human Melanoma-Associated Mast Cells Display a Distinct Transcriptional Signature Characterized by an Upregulation of the Complement Component 3 That Correlates With Poor Prognosis. Frontiers in immunology. 2022 May 20;13.
12. Ribatti D. New insights into the role of mast cells as a therapeutic target in cancer through the blockade of immune checkpoint inhibitors. Front Med (Lausanne). 2024 Feb 28;11:1373230.
13. Nardone B, Martini M, Busam K, Ashfaq Marghoob, West DP, Gerami P. Integrating clinical/dermatoscopic findings and fluorescence in situ hybridization in diagnosing melanocytic neoplasms with less than definitive histopathologic features. Journal of the American Academy of Dermatology. 2012 Jun 1;66(6):917–22.

Conflict of interest
NONE DECLARED

Correspondance address: Cristina-Raluca Mihulecea
Sibiu, Al. Șelimbăr Nr. 2A, e-mail: cristina.jitian@yahoo.com