Reemergence of neural crest stem cell-like states in melanoma during disease progression and treatment

Johanna Diener | Lukas Sommer

Abstract
Melanoma is the deadliest of all skin cancers due to its high metastatic potential. In recent years, advances in targeted therapy and immunotherapy have contributed to a remarkable progress in the treatment of metastatic disease. However, intrinsic or acquired resistance to such therapies remains a major obstacle in melanoma treatment. Melanoma disease progression, beginning from tumor initiation and growth to acquisition of invasive phenotypes and metastatic spread and acquisition of treatment resistance, has been associated with cellular dedifferentiation and the hijacking of gene regulatory networks reminiscent of the neural crest (NC)—the developmental structure which gives rise to melanocytes and hence melanoma. This review summarizes the experimental evidence for the involvement of NC stem cell (NCSC)-like cell states during melanoma progression and addresses novel approaches to combat the emergence of stemness characteristics that have shown to be linked with aggressive disease outcome and drug resistance.

KEYWORDS
cancer, development, drug resistance, invasion, melanoma, neural crest stem cells (NCSCs), tumor initiation

INTRODUCTION

The neural crest (NC) is a transient and multipotent stem cell population arising at the dorsal neural tube at early stages of vertebrate development. Neural crest stem cells (NCSCs) disseminate into the embryo to differentiate into an array of cell lineages with remarkably different functions. Among the adult NC derivatives are cartilage and bone structures in the head, the outflow tract of the heart, enteric nervous system cells in the gut, neuronal and non-neuronal cells of the peripheral nervous system, melanocytes and others.

While most of the NC progeny have acquired a fully differentiated fate in the adult, cells with NCSC-like characteristics have been isolated from several adult mouse tissues as well as human skin and dental pulp. Even though the isolated cells possessed multipotent capacities in vitro and could give rise to different NC lineages, the question remains whether NCSC-like cells derived from adult tissues really represent a maintained embryonic stem cell niche or whether these cells reacquire stem cell features due to isolation procedures and cultivation in vitro.

The reemergence of NCSC-like cell states in vivo has been observed and studied in the context of pathological and physiological conditions such as carcinogenic transformations of NC-derived adult tissues or wound healing, respectively. Murine tissue injury models revealed that NC-derived Schwann cells (peripheral glia) dedifferentiate after injury and reacquire a progenitor-like cell state by downregulating genes crucial for the myelination machinery and upregulating NCSC-associated factors such as the neurotrophin receptor CD271 (also p75NTR, NGFR). Injury-activated glial cells were further shown to crucially assist regeneration of full-thickness skin wounds or amputated digit tips.
Furthermore, previous reports revealed that Schwann cell precursors (SCPs) during embryonic development harbor NC cell features and are able to generate a variety of neural and non-neural cell types.\textsuperscript{15,16} Likewise, carcinogenic lesions of adult NC-derived tissues such as melanoma or neuroblastoma can both present with cells expressing NCSC-associated factors that are not normally expressed in the healthy adult tissue.\textsuperscript{19-21} This review focuses on melanoma, wherein the aberrant regulation or expression of developmental NCSC genes or pathways is associated with different aspects of malignancy (Figure 1), such as tumor initiation and sustained tumor growth,\textsuperscript{22-25} promotion of metastatic spread,\textsuperscript{26} as well as resistance to therapies\textsuperscript{27-30} and immune evasion.\textsuperscript{31,32}

2 | DEVELOPMENTAL NCSC GENES REGULATE MELANOMA GROWTH AND HOMEOSTASIS

Cutaneous malignant melanoma, the most aggressive skin cancer,\textsuperscript{33} can present with astonishing heterogeneity, ranging from cells expressing typical melanocyte differentiation genes to gene sets typical for other NC-derived cell lineages like neurons or glia, among others.\textsuperscript{34-36} The origin of such heterogeneous tumors has been heavily debated and research was carried out on the expression of factors reminiscent of stem cells, which equips melanoma cells with renewal capacities in vitro and tumor formation potential in vivo.\textsuperscript{22,37,38} For instance, the neurotrophin receptor CD271, which has been used for isolation of rat NCSCs with multipotent capacities in vitro,\textsuperscript{14} was found reexpressed in a subset of melanoma cells that were able to initiate tumor formation when grafted into immunocompromised mice, while CD271-negative cells lacked such potential.\textsuperscript{22}

**Significance statement**

This review summarizes the experimental evidence for reemergence of neural crest stem cell (NCSC)-like cells in melanoma, which hijack embryonic programs to acquire advantages regarding tumor initiation and growth, metastatic spread, and eventually therapy resistance. Furthermore, the authors discuss preclinical efforts to specifically target NCSC-like melanoma cells to combat drug resistance, which is a major goal in the field and will hopefully soon improve melanoma therapy for patients.

---

**FIGURE 1** Reacquisition of neural crest stem cell (NCSC)-like characteristics in melanoma and its implications. Embryonic NCSCs (dark green) arise at the developing dorsal neural tube and migrate into the whole embryo to form different tissues such as craniofacial bone and cartilage, enteric and peripheral nervous system cells (light grey) and, amongst many others, also cells of the melanocyte lineage, specifically melanocyte stem cells (MeSCs), melanoblasts and melanocytes (brown). Due to malignant transformations, those cells can progress into melanoma. Different studies have shown that some melanoma cells (light green) can hijack embryonic NCSC programs, bestowing them with different advantageous characteristics. Melanoma cells with reacquired NCSC features have been associated with the ability to form novel tumors and sustain growth, increased cell invasiveness and metastasis formation, as well as the ability to resist different melanoma therapies and evade immune surveillance.
cycle regulatory networks to allow tumor initiation and maintenance.

Yet, the mystery of how human melanoma arises in vivo remains to be unraveled. While some studies report that SOX2 promotes tumor initiation and maintained tumor growth. The transcription factor (TF) SRY-related HMG-box 10 (SOX10), for instance, which regulates the melanocytic and glial lineages derived from the NC, was found highly expressed in melanocytic nevi and malignant melanoma. Upon depletion of SOX10 in a transgenic NRASQ61K-mutant, Ink4a-deficient murine melanoma model, tumor growth was diminished, and silencing of SOX10 in human melanoma cells drastically reduced the number of cells expressing CD271 and completely abolished tumor growth in vivo.

There has been much debate recently about whether the cell at the origin of melanoma indeed represents a stem cell-like cell. In fact, the above-mentioned studies somewhat stand in contrast to reports claiming that instead of cells with stem cell features, fully differentiated melanocytes can give rise to melanoma. For instance, it was shown that BRAFV600E-deficient fully differentiated and melanin-producing melanocytes could give rise to melanoma.

Yet another theory on the origin of melanoma argues that McSCs residing in the bulge of the hair follicle, rather than differentiated melanocytes, could stand at the origin of melanoma primary tumor formation. Moreover, given that adult Schwann cells have the capacity of dedifferentiation in vivo and that SCPs are able to generate melanocytes, it is conceivable that some melanoma might derive from the peripheral glial lineage.

Several animal models have allowed melanoma induction with specific cues followed by in-depth analysis and lineage tracing to understand disease initiation and progression. However, such analyses remain challenging in the context of human disease. While single-cell studies of patient-derived melanoma allow unprecedented insights into heterogeneous disease evolution, an alternative approach to tackle melanoma origin as such, could be the artificial induction of melanoma in melanocyte lineage cells derived from human embryonic stem cells followed by lineage tracing and single-cell characterization. Yet, the mystery of how human melanoma arises in vivo remains to be unraveled.

### 3 | NCSC-LIKE CELLS CAN INDUCE INVASION AND METASTATIC SPREAD OF MELANOMA

Apart from inducing or maintaining melanoma tumor growth, the reacquisition of NCSC-like characteristics has also been associated...
| Melanoma implication | Factor (function) | Detailed function in melanoma | References | NCSC function | References |
|----------------------|------------------|-------------------------------|------------|---------------|------------|
| Tumor initiation/growth | SOX10 (TF) | Broadly expressed in human nevi and melanomas. Depletion leads to abolished tumor formation in a NRAS\textsuperscript{G61K} Ink4a-deficient mouse model, associated with reduced numbers of CD271\textsuperscript{+} cells. | 24 | Specifies murine NCSCs and melanocytic and glial lineages. | 39,40 |
|                      | YY1 (TF) | Haploinsufficiency is enough to prevent melanoma initiation in a NRAS\textsuperscript{G61K} Ink4a-deficient mouse model. Regulates shared metabolic and translational pathways in neural crest and melanoma. | 25 | Essential for early murine NC development and the adult melanocyte lineage. | 25 |
|                      | CD271/NGFR/p75\textsuperscript{NTR} (receptor tyrosine kinase) | Single CD271\textsuperscript{+} melanoma patient-derived cells can form tumors (with the heterogeneity of the parental tumor) upon grafting into immunocompromised mice, while CD271\textsuperscript{-} cells cannot. Inhibition of CD271 in human melanoma cells reduces their tumor initiation potential. | 22,41 | Used to isolate mammalian NCSCs that were multipotent in vitro | 14 |
|                      | EZH2 (histone methyl transferase) | Upregulated in human malignant melanoma compared to melanocytes. Depletion in a NRAS\textsuperscript{G61K} Ink4a-deficient mouse model inhibits melanoma growth. | 42 | Controls differentiation of NC-derived mesenchymal lineages (bone and cartilage) | 43 |
|                      | DHODH (pyrimidine metabolism) | Transcriptional elongation of genes crucial for melanogenesis. | 44 | Transcriptional elongation of neural crest developmental genes. | 44 |
|                      | DDX21 (RNA helicase) | Controls transcriptional elongation (after nucleotide shortage-induced stress). | 45 | Controls transcriptional elongation. | 45 |
|                      | Crestin (unknown) | Marks tumor-initiating cells in a BRAF\textsuperscript{V600E} p53-deficient zebrafish melanoma model. | 23 | mRNA widely expressed in zebrafish NCSCs | 46 |
| Phenotype switch/invasion | MSX1 (TF) | Induces phenotypic switching (E-cadherin\textsuperscript{high}, nonmigratory toward ZEB1\textsuperscript{high}, invasive) in melanoma. Depletion reduces liver metastasis after tail vain injection of human melanoma cells into immunocompromised mice. | 47 | NC induction in xenopus | 48 |
|                      | Twist1/Zeb1 (TF) | NRAS/BRAF activation in melanocytes leads to upregulation of EMT TFs including TWIST1 and ZEB1, dedifferentiation and neoplastic transformation of melanocytes. | 49 | Twist1: NC specifier; delamination of cranial NC; cell fate decision within cardiac NC Zeb1: upregulated by Zeb2, essential for melanocyte migration and differentiation. | 50-52 |
|                      | FOXD3/PAX3 (TF) | FOXD3 and PAX3 drive CXCR4 expression in melanoma, which was shown to promote melanoma metastasis formation. | 53,54,55 | FOXD3: NC specifier PAX3: expressed in neural plate border | 56,57 |
|                      | CD271 (receptor tyrosine kinase) | Associated with increased metastasis in patients. Transient overexpression induces a reversible phenotype switch in vitro and increased metastatic potential of human melanoma cells grafted onto immunocompromised mice. | 35,26 | | |
with later stages of melanoma progression, such as invasiveness and metastatic spread. To become invasive and disseminate from the primary tumor, cancer cells are thought to undergo an epithelial to mesenchymal transition (EMT), rendering them with decreased cell-to-cell contacts, increased motility, and an increased potential to remodel extracellular matrix components.

Although, by definition, classical EMT is a process associated with epithelial cancers, which is not the case for melanoma, melanoma cells can undergo an EMT-like process called “phenotype switching,” where cells transform from a high proliferative/low invasive to a low proliferative/high invasive phenotype. Even though it has been reported that melanoma invasion and metastasis can progress independently of the ‘classical’ phenotype switching model, EMT-like phenotype switching is thought to be a crucial driver of melanoma invasiveness and metastasis formation.

Migratory NCSCs delaminating from the neural tube to migrate out into the embryo are a paradigm example for EMT during embryonic development. Intriguingly, regulatory genes responsible for developmental NC EMT are reexpressed in adult malignant melanoma, including members of the Snail, Zeb, and Twist families (Table 1). Caramel et al showed that upon NRAS/BRAF activation in melanocytes, the EMT TFs TWIST1 and ZEB1 were upregulated and induced dedifferentiation and neoplastic transformation. They also showed that this EMT-TF signature, when found in late-stage melanoma patients, correlated with poor prognosis. Similarly, transforming growth factor beta also acts as a potent inducer of melanoma phenotype switching, while playing a crucial role in melanoma progression.

### Table 1 (Continued)

| Melanoma implication | Factor (function) | Detailed function in melanoma | References | NCSC function | References |
|----------------------|-------------------|------------------------------|------------|---------------|------------|
| Drug Resistance      | FOXD3 (TF)        | FOXD3 upregulates ERBB3, leading to BRAFi resistance in vitro and in vivo. | 58         | ERBB3: NC differentiation and dev. of sympathetic nervous system | 59         |
|                      | ERBB3 (receptor tyrosine kinase) | | | | |
|                      | CD271 (receptor tyrosine kinase) | NGFR' AXL' melanoma patient cells represent a dormant, MAPKi-resistant cell population. Long-term (3 weeks) BRAFi treatment leads to emergence of a drug-tolerant or drug-resistant NC-like cell state in vitro. | 60,61 | | |
|                      | RXRG (nuclear receptor) | Minimal residual disease in a BRAFi/MEKi-tolerant PDX model represents as dedifferentiated melanoma (NGFR’ RXRG’ AQP1’ GFRA’). | 28         | Expressed in migrating cranial chick NC cells. | 62         |
| Immune Evasion       | CD271 (receptor tyrosine kinase) | TNFα induces dedifferentiation of melanoma cells (NGFR[60]) and resistance to adaptive T-cell therapy in a murine model of adoptive cell transfer therapy. Long-term exposure of patient-derived melanoma cells to antigen-specific cytotoxic T cells leads to enrichment of NGFR[60] cells, which are refractory to T cells as well as to BRAF/MEKi | 32,31 | | |
|                      | EZH2 (histone methyl transferase) | Intratumoral TNFα and T-cell accumulation induce Ezh2 in melanoma cells originating from NRAS[61] Ink4a-loss or B16 F10 murine models, leading to loss of immunogenicity. | 63         | | |

Abbreviations: MEK, mitogen-activated extracellular signal-regulated kinase; NC, neural crest; NCSC, neural crest stem cell; SOX10, SRY-related HMG-box 10; TF, transcription factor; YY1, Ying Yang 1. Transgenic animal models: NRAS[61] Ink4a-deficient mouse model, Tyr:Nras[61] Cdkn2a-/-; BRAF[66] p53-deficient zebrafish, mitfa:BRAF[66] p53-/-.
physiological role in NC development by providing signaling cues for migration and differentiation into several lineages.90–92

Apart from an invasive and migratory potential, epithelial cancer cells undergoing EMT have been associated with the acquisition of stem cell-like features.93,94 Similarly, melanoma phenotype switching has been associated with the reemergence of signatures similar to NCSC regulatory networks.97 (Table 1). Expression of the NCSC-associated factors CD271 and SOX1039,40 in human melanoma correlates with high metastatic potential and worse patient prognosis.35 (Table 1). Furthermore, Msh homeobox 1 (MSX1), which specifies the NC at the neural border of zebrafish and xenopus,48 when upregulated in melanoma, leads to dedifferentiation of melanoma cells, which upregulate NCSC-associated factors such as CD271 and induce a phenotype switch toward increased cell migration.47 (Table 1). Vice versa, silencing of MSX1 reduces liver metastasis of tail vein-injected human melanoma cells in mice.47 Also the zebrafish and murine NC specifier FOXD3 together with PAX3, which is expressed at the neural plate border,56,57 have been shown to induce human melanoma invasiveness by directly regulating CXCR4,53 which in turn regulates melanoma metastasis formation.54,55 (Table 1). Finally, even though a zebrafish study suggested otherwise,95 ectopic overexpression of CD271 induces a phenotype switch in human melanoma cells, ultimately leading to an increased metastatic potential of human melanoma cells grafted into immunocompromised mice.26 (Table 1). These findings revealed a functional involvement of single factors reminiscent of NCSCs in melanoma disease progression. Whether, in general, the reemergence of a broader NCSC signature is functionally implicated in melanoma metastasis formation remains to be elucidated.

4 | DEDIFFERENTIATED MELANOMA CELLS DISPLAY RESISTANCE TO DIFFERENT THERAPIES

While traditionally the most common therapy for melanoma has been surgical removal of primary tumors plus radiation and chemotherapy,96 the advent of immune and targeted therapies significantly improved the survival rate, especially of patients with metastatic melanoma.33 Targeted therapies for melanoma are mostly directed against the serine/threonine kinase BRAF or the mitogen-activated extracellular signal-regulated kinase (MEK), leading to inhibition of the mitogen-activated protein kinase (MAPK) pathway, an oncogenic pathway mutated and constitutively active in most melanomas.97,98 Immunotherapies on the other hand aim at boosting the antitumoral activity of cytotoxic T lymphocytes (CTLs) to combat melanoma.29 However, one of the major remaining challenges is the acquisition or preexistence of melanoma cells resistant to such therapies.99–102 which ultimately lead to relapse.

Resistance to different melanoma therapies has been associated with cells undergoing phenotype switching and lacking pigmentation-related differentiation genes while expressing genes reminiscent of NC development.28–30,32,104,105 Specifically, MAPK pathway inhibition was shown to promote the de novo generation or expansion of subpopulations of melanoma cells expressing NCSC-associated factors like CD27128,106 or the NC specifier gene FOXD3.58 In addition, targeted therapy led to increased expression of genes linked to invasiveness like the receptor tyrosine kinase AXL.106,107 (Table 1). NCSC-like melanoma cell subpopulations were further reported to contribute to minimal residual disease and, ultimately, to disease relapse.28,108 Conversely, another study showed that melanoma cells expressing the melanocyte differentiation gene Dopachrome tautomerase (DCT, also TYRP2) were intrinsically resistant to BRAF inhibition.109 However, increasing evidence supports the hypothesis that it is particularly the dedifferentiated melanoma cell population, expressing genes reminiscent of NCSCs, that can resist different treatments, including immunotherapies.

Along that line, a recent study showed that long-term exposure of patient-derived melanoma cells to antigen-specific (MART1) T cells led to an enrichment of CD271-high melanoma cells, which showed increased resistance to cytotoxic T cells (which recognized differentiation and non-differentiation antigens), as well as to BRAF and MEK inhibitors31 (Table 1). In line with these findings, another study revealed that in a mouse adoptive T cell therapy model, tumor necrosis factor alpha (TNF-α)-induced inflammation led to dedifferentiation of patient-derived transplanted melanoma by upregulation of CD271 and downregulation of melanocyte-specific antigens, which resulted in reduced tumor recognition by infiltrating T cells32 (Table 1). Furthermore, while Bosshuizen et al31 interfered with CD271 upregulation to combat T cell resistance and relapse in their preclinical model via an unspecific heat shock protein inhibitor, another study developed a CD271-specific monoclonal humanized antibody to counteract CD271 function.110 The authors showed that treatment of CD271-positive human melanoma grafts in NOD/scid mice with this CD271 antibody in combination with natural killer or peripheral blood mononuclear effector cells achieved a significant antitumor effect.110 Whether such a CD271-specific antibody could combat therapy resistance of melanoma tumors toward targeted or immune therapy remains to be answered, but previous findings appear to support such an approach.28,31,32

Immunotherapy-induced T-cell accumulation and TNFα have also been shown to induce other factors reminiscent of NC development in melanoma, such as the histone methyltransferase Ezh2, which regulates mesenchymal fates during murine NC development.43 Ezh2 upregulation in melanoma led to reduced immunogenicity of B16 F10 or NrasQ61K-mutant Ink4a-null mice tumors autologously grafted onto C57BL/6 mice, while pharmacological inhibition of Ezh2 attenuated this effect and synergized with anti-CTLA-4 and IL-2 immunotherapies in mice63 (Table 1).
TARGETING THE REEMERGENCE OF NCSC-LIKE MELANOMA STATES

In line with NCSC-like cell states observed in melanoma during disease progression and treatment, increasing evidence supports approaches targeting melanoma cells that have hijacked developmental programs. Indeed, several preclinical studies have succeeded in targeting or inhibiting the emergence of dedifferentiated melanoma cells to combat therapy resistance (Figure 2). For instance, inhibiting CD271 in melanoma cells restored their susceptibility to BRAF inhibitors.27 Likewise, suppressing the emergence of dedifferentiated, NCSC-like melanoma cells upon MEK and BRAF inhibition (MEKi and BRAFi) interfered with resistance formation in vivo.28 Specifically, this elegant study showed that a set of NCSCs genes, such as the Retinoid X receptor gamma (RXRG), which is expressed in chicken NCSCs,62 was induced by MEKi and BRAFi treatment, and that pharmacological inhibition of RXRG prevented disease relapse of patient-derived melanoma in immunocompromised mice28 (Figure 2). Yet another study used a cytotoxic antibody approach to target AXLhigh melanoma cells resistant to MEKi and BRAFi, which led to decreased melanoma cell survival. Sáez-Ayala et al111 achieved to circumvent drug resistance by forced differentiation of melanoma cells due to treatment with methotrexate (MTX), which induced the expression of the melanocyte differentiation marker MITF and inhibited invasiveness. This drug was further combined with the cytotoxic prodrug TMECG, activated by tyrosinase (a target of MITF), which is expressed in differentiated melanocytes.
Furthermore, targeted as well as immunotherapy have been shown to induce drug resistance, along with acquisition of NCSC-like gene expression programs, that lead to an increased sensitivity to ferroptosis, a type of programmed cell death. The study authors subsequently managed to inhibit melanoma dedifferentiation and therapy resistance by addition of ferroptosis-inducing drugs (Figure 2).

The opposite approach of pushing melanoma into a fully differentiated cell fate to circumvent resistance formation, has been achieved in vitro through application of methotrexate (MTX), which activates the expression of microphthalmia-induced TF (MITF), a key regulator of differentiated melanocytes. MTX treatment further increased melanoma cell susceptibility to a cytotoxic prodrug (TMECG), activated by tyrosinase, a target of MITF and expressed by fully differentiated melanocytes (Figure 2).

All in all, these studies have demonstrated that interference with melanoma dedifferentiation or, vice versa, the promotion of a fully differentiated melanoma state can yield increased drug susceptibility and prevention of disease relapse in preclinical models, which make this a highly promising approach for patient therapy.

6 | CONCLUDING REMARKS

Several approaches to target the reemergence of NCSC-like cell states in treatment-resistant melanomas (Figure 2) have shown great promise in preclinical settings. However, further in-depth studies and proper characterization of such NCSC-like melanoma cell states are needed to unravel the exact nature of gene regulatory networks that lead to the most therapy-resilient, and hence aggressive, tumors. Unfortunately, inter-patient and intratumoral heterogeneity has always posed a substantial challenge in melanoma treatment and also complicates the identification of exact NCSC-like programs emerging within melanoma patients.

Currently, most studies performed on NCSC-like cells in melanoma and cited within this review are preclinical studies performed on human material in vitro or in murine models, where NC-reminiscent factors were shown to be crucial for tumor formation and disease progression in genetically engineered melanoma models as well as in patient-derived xenograft models. However, extensive analyses of patient materials, which are important to support the clinical relevance of the above discussed findings, are often missing due to limited access to samples reflecting specific stages of melanoma progression. Also, some of the first in-depth single-cell analyses of patient tissues have been single case reports rather than studies involving big patient cohorts. Therefore, the possibility remains that the reemergence of aggressive, therapy-resistant NCSC-like cell states observed in preclinical models does not or not always occur during disease progression in human melanoma patients and that metastatic disease and therapy resistance could still be established by alternative pathways.

Another remaining question concerns whether the mutational landscape predisposes melanoma subtypes toward the potential for dynamic remodeling into NCSC-like cell states. For now, it is unclear whether some of the most frequent melanoma mutations, namely, BRAF\(^{V600E}\) and NRAS\(^{Q61K}\), preferentially favor the reemergence of NCSC-like cell states. Work by Zon and colleagues has shown that in zebrafish, BRAF\(^{V600E}\)-mutated melanomas activate a NCSC progenitor program essential for melanoma initiation, while in NRAS\(^{Q61K}\)-mutated melanomas, such a NCSC signature did not emerge at early stages of disease but only after transformation into malignant melanoma. Whether this discrepancy is reflective of the human disease physiology remains unclear, since tumor initiation and onset of invasion and metastatic spread cannot be properly monitored or modeled in humans. Furthermore, most preclinical studies that have associated NCSC-like melanoma subpopulations with resistance to therapy have addressed BRAF inhibitor resistance and accordingly used mostly BRAF-mutated melanoma material, leading to a bias toward BRAF vs NRAS-mutated material under investigation.

In conclusion, future studies including human material from bigger, more representative patient cohorts and collected at different time points of disease progression, specifically also during response to therapies, are needed to address the relevance of NCSC-like melanoma cell states in humans. Hopefully, further single-cell studies will allow us to answer whether NCSC-reminiscent melanoma subpopulations indeed emerge in patients and whether they substantially interfere with melanoma treatment. This could open promising new avenues for designing novel therapies.

ACKNOWLEDGMENT

We thank Rishika Pandya for critical reading of this manuscript.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

J.D., L.S.: wrote this manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Johanna Diener https://orcid.org/0000-0002-7733-5904

Lukas Sommer https://orcid.org/0000-0002-1143-7908

REFERENCES

1. Baggiolini A, Varum S, Mateos JM, et al. Premigratory and migratory neural crest cells are multipotent in vivo. Cell Stem Cell. 2015;16(3):314-322. https://doi.org/10.1016/j.stem.2015.02.017.

2. Bronner-Fraser M, Fraser SE. Cell lineage analysis reveals multipotency of some avian neural crest cells. Nature. 1988;335:161-164. https://doi.org/10.1038/335161a0.
3. Le Douarin, N., & Kalcheim, C. (1999). The Neural Crest. Cambridge, England: Cambridge University Press. https://doi.org/10.1017/CBO9780511897948

4. Szabó A, Mayor R. Mechanisms of neural crest migration. Annu Rev Genet. 2018;52(1):43-63. https://doi.org/10.1146/annurev-genet-120417-031559.

5. Etchevers HC, Dupin E, Le Douarin NM. The diverse neural crest: from embryology to human pathology. Development (Cambridge). 2019;146(2):dev169821. https://doi.org/10.1242/dev.169821.

6. Simões-Costa M, Bronner ME. Establishing neural crest identity: a gene regulatory recipe. Development. 2015;142(1):242-257. https://doi.org/10.1242/dev.105445.

7. Donalek RL, Diegelmann RF, Hecht HS. The Wound Healing Process. 2002;6(2):111-132. https://doi.org/10.1016/S1527-2526(02)00014-4.

8. Fernandes KJL, McKenzie IA, Mill P, et al. A dermal niche for multipotent skin-derived precursors from human skin. Cell Stem Cell. 2005;23(6):727-737. https://doi.org/10.1016/j.stem.2004-0134.

9. Sieber-Blum M, Grim M, Hu YF, Szeder V. Pluripotent neural crest stem cells in the adult hair follicle. Dev Dyn. 2004;222(2):334-348. https://doi.org/10.1002/dvdy.20122.

10. Toma JG, McKenzie IA, Mill P, et al. A dermal niche for multipotent skin-derived precursor cells. Nat Cell Biol. 2004;6:1082-1093. https://doi.org/10.1038/ncc1181.

11. Kameneva P, Kastriti ME, Adameyko I. Neuronal lineages derived from embryonic neural crest cells. Dev Dyn. 2005;234(2):462-473. https://doi.org/10.1002/dvdy.22012.

12. Wong CE, Paratore C, Dours-Zimmermann MT, et al. Neural crest-derived cells with stem cell features can be traced back to multiple lineages in the adult skin. J Cell Biol. 2006;175(6):1005-1015. https://doi.org/10.1083/jcb.20060602.

13. Al-Zer H, Apel C, Heiland M, et al. Enrichment and Schwann cell differentiation of neural crest-derived dental pulp stem cells. In Vivo. 2015;29(3):319-326.

14. Stemple DL, Anderson DJ. Isolation of a stem cell for neurons and glia from the mammalian neural crest. Cell. 1992;71:973-985.

15. Furlan A, Adameyko I. Schwann cell precursor: a neural crest cell in early development to adulthood. Dev Genet. 2012;36(1):83-95. https://doi.org/10.1016/j.ydbio.2012.02.035.

16. Fernandes KJL, McKenzie IA, Mill P, et al. A dermal niche for multipotent skin-derived precursor cells. Nat Cell Biol. 2004;6:1082-1093. https://doi.org/10.1038/ncc1181.

17. Simões-Costa M, Bronner ME. Establishing neural crest identity: a gene regulatory recipe. Development. 2015;142(1):242-257. https://doi.org/10.1242/dev.105445.

18. Wong CE, Paratore C, Dours-Zimmermann MT, et al. Neural crest-derived cells with stem cell features can be traced back to multiple lineages in the adult skin. J Cell Biol. 2006;175(6):1005-1015. https://doi.org/10.1083/jcb.20060602.

19. Al-Zer H, Apel C, Heiland M, et al. Enrichment and Schwann cell differentiation of neural crest-derived dental pulp stem cells. In Vivo. 2015;29(3):319-326.

20. Meisler MH, Lindsell J, Wilson AC, et al. Isolation of a stem cell for neurons and glia from the mammalian neural crest. Cell. 1992;71:973-985.

21. Kaufman CK, Mosimann C, Fan ZP, et al. A zebrafish melanoma model reveals emergence of neural crest identity during melanoma initiation. Science. 2016;351(6272):aad2197. https://doi.org/10.1126/science.aad2197.

22. Shakhova O, Zingg D, Schaefer SM, et al. Sox10 promotes the formation and maintenance of giant congenital naevi and melanoma. Nat Cell Biol. 2012;14(8):882-889. https://doi.org/10.1038/nclb2535.

23. Varum S, Baggioni A, Zurkirchen L, et al. Yin Yang 1 orchestrates a metabolic program required for both neural crest development and melanoma formation. Cell Stem Cell. 2019;24(4):637-653.e9. https://doi.org/10.1016/j.stem.2019.03.011.

24. Restivo G, Diener J, Cheng PF, et al. The low affinity neurotrophin receptor CD271 regulates phenotype switching in Melanoma. Nat Commun. 2017;8(1):1-16. https://doi.org/10.1038/s41467-017-01573-6.

25. Lehraìk A, Cerezo M, Rouaud F, et al. Increased CD271 expression by the NF-kB pathway promotes melanoma cell survival and drives acquired resistance to BRAF inhibitor vemurafenib. Cell Discov. 2015;1:15030. https://doi.org/10.1038/celldisc.2015.30.

26. Rambow F, Rogiers A, Marin-Bejar O, et al. Toward minimal residual disease-directed therapy in melanoma. Cell. 2018;174(4):843-855. e19. https://doi.org/10.1016/j.cell.2018.06.025.

27. Schatton T, Scolyer RA, Thompson JF, Mihm MC. Tumor-infiltrating lymphocytes and their significance in melanoma prognosis. Methods Mol Biol. 2014;1102:287-324. https://doi.org/10.1007/978-1-62703-727-3_16.

28. Shaffer SM, Dunagin MC, Torborg SR, et al. Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance. Nature. 2017;546(7658):431-435. https://doi.org/10.1038/nature22794.

29. Boshuizen J, Vredevoogd DW, Krijgsman O, et al. Reversal of pre-existing NGFR-driven tumor and immune therapy resistance. Nat Commun. 2020;11(1):1-13. https://doi.org/10.1038/s41467-020-17739-8.

30. Landsberg J, Kohli Meyer J, Renn M, et al. Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. Nature. 2012;490(7420):412-416. https://doi.org/10.1038/nature11538.

31. Lo JA, Fisher DE. The melanoma revolution: From UV carcinogenesis to a new era in therapeutics. Science. 2014;346(6212):945-949. https://doi.org/10.1126/science.1253735.

32. Banerjee SS, Eyden B. Divergent differentiation in malignant melanomas: a review. Histopathology. 2008;52(2):119-129. https://doi.org/10.1111/j.1365-2559.2007.02823.x.

33. Civenni G, Walter A, Kobot N, et al. Human CD271-positive melanoma stem cells associated with metastasis establish tumor heterogeneity and long-term growth. Cancer Res. 2011;71(8):3098-3109. https://doi.org/10.1158/0008-5472.CAN-10-3997.

34. Ennen M, Keime C, Kobi D, et al. Single-cell gene expression signatures reveal melanoma cell heterogeneity. Oncogene. 2015;34:3251-3263. https://doi.org/10.1038/onc.2014.426.

35. Beck B, Blanpain C. Unravelling cancer stem cell potential. Nat Rev Cancer. 2013;13(10):727-738. https://doi.org/10.1038/nrc3597.

36. Schatton T, Frank MH. Cancer stem cells and human malignant melanoma. Pigment Cell Melanoma Res. 2008;21(1):39-55. https://doi.org/10.1111/j.1755-148X.2007.00427.x.

37. Kim J, Lo L, Dormand E, Anderson DJ. SOX10 maintains multipotency and inhibits neuronal differentiation of neural crest stem cells. Neuron. 2003;38:17-31. https://doi.org/10.1016/S0896-6273(03)00163-6.

38. Paratore C, Goerich DE, Suter U, Wegner M, Sommer L. Survival and glial fate acquisition of neural crest cells are regulated by an interplay between the transcription factor Sox10 and extrinsic combinatorial signaling. Development. 2001;128(20):3949-3961.
41. Redmer T, Welte Y, Behrens D, et al. The nerve growth factor receptor CD271 is crucial to maintain tumorigenicity and stem-like properties of melanoma cells. PLoS One. 2014;9(5):e92596. https://doi.org/10.1371/journal.pone.0092596.

42. Zingg D, Debbache J, Schaefer SM, et al. The epigenetic modifier EZH2 controls melanoma growth and metastasis through silencing of distinct tumour suppressors. Nat Commun. 2015;6(May 2014):1-17. https://doi.org/10.1038/ncomms7051.

43. Schwarz D, Varum S, Zemke M, et al. EZH2 is required for neural crest-derived cartilage and bone formation. Development (Cambridge). 2014;141(4):867-877. https://doi.org/10.1242/dev.094342.

44. White RM, Cech J, Ratanasirintrawoot S, et al. DHODH modulation of nucleotide stress responses in neural crest and melanoma cells. Cell Bio. 2020;22(4):372-379. https://doi.org/10.1038/s41556-020-0493-0.

45. Luo R, An M, Arduini BL, Henion PD. Specific pan-neural crest expression of zebrafish crestin throughout embryonic development. Dev Dyn. 2001;220(2):169-174. https://doi.org/10.1002/1097-0177(20009999<::AID-DVDY1097>3.0.CO;2-1).

46. Heppt MV, Wang JX, Hristova DM, et al. MSX1-induced neural crest-like reprogramming promotes melanoma progression. J Invest Dermatol. 2018;138(1):141-149. https://doi.org/10.1016/j.jid.2017.05.038.

47. Tribulo C, Aybar MJ, Nguyen VH, Mullins MC, Mayor R. Regulation of Msa genes by a Bmp gradient is essential for neural crest specification. Development. 2003;130(26):6441-6452. https://doi.org/10.1242/dev.00878.

48. Caramel J, Papadogeorgakis E, Hill L, et al. A switch in the expression of embryonic EMT-inducers drives the development of malignant melanoma. Cancer Cell. 2013;24(4):466-480. https://doi.org/10.1016/j.ccell.2013.08.018.

49. Bildsoe H, Loebel DAF, Jones VJ, Chen YT, Behringer RR, Tam PPL. Requirement for Twist1 in frontonasal and skull vault development in the mouse embryo. Dev Bio. 2009;331(2):176-188. https://doi.org/10.1016/j.ydbio.2009.09.034.

50. Vincentz JW, Firulli BA, Lin A, Spencer DB, Howard MJ, Firulli AB. Twist1 controls a cell-specificiation switch governing cell fate decisions within the cardiac neural crest. PLoS Genet. 2013;9(3):e1003405. https://doi.org/10.1371/journal.pgen.1003405.

51. Denecker G, Vandamme N, Akay Ö, et al. Identification of a ZEB2-MITF-ZEB1 transcriptional network that controls melanogenesis and melanoma progression. Cell Death Differ. 2014;21(8):1250-1261. https://doi.org/10.1038/cdd.2014.44.

52. Kubic JD, Lui JW, Little EC, et al. PAX3 and FOXD3 promote CXCR4 expression in melanoma. J Biol Chem. 2015;290(36):21901-14. https://doi.org/10.1074/jbc.M115.670976.

53. Bartolomé RA, Galvez BG, Longo N, et al. Stromal cell-derived factor-1A promotes melanoma cell invasion across basement membranes involving stimulation of membrane-type 1 matrix metalloproteinase and Rho GTPase activities. Cancer Res. 2004;64(7):2534-2543.

54. Murakami T, Maki W, Cardones AR, et al. Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. Cancer Cell. 2002;1:7328-7334.

55. Betancur P, Bronner-Fraser M, Saaka-Spengler T. Assembling neural crest regulatory circuits into a gene regulatory network. Annu Rev Cell Dev Biol. 2010;26:581-603. https://doi.org/10.1146/annurev.cellbio.042308.113245.ASSEMBLING.
75. Schaefer SM, Segalada C, Cheng PF, et al. Sox2 is dispensable for primary melanoma and metastasis formation. Oncogene. 2017;36(31):4516-4524. https://doi.org/10.1038/onc.2017.55.

76. Johansson JA, Marie KL, Lu Y, et al. PRL3-DDX21 transcriptional control of endolyosomal genes restricts melanocyte stem cell differentiation. Dev Cell. 2020;54(3):317-332.e9. https://doi.org/10.1016/j.devcel.2020.06.013.

77. Köhler C, Nittner D, Rambow F, et al. Mouse cutaneous melanoma induced by mutant Braf arises from expansion and dedifferentiation of mature pigmented melanocytes. Cell Stem Cell. 2017;21(5):679-693.e6. https://doi.org/10.1016/j.stem.2017.08.003.

78. Moon H, Donahue LR, Choi E, et al. Melanocyte stem cell activation and translocation initiate cutaneous melanoma in response to UV exposure. Cell Stem Cell. 2017;21(5):665-678.e6. https://doi.org/10.1016/j.stem.2017.09.001.

79. Sun Q, Lee W, Mohri Y, et al. A novel mouse model demonstrates that oncogenic melanocyte stem cells engender melanoma resembling human disease. Nat Commun. 2019;10(1):1-16. https://doi.org/10.1038/s41467-019-12733-1.

80. Adameyko I, Lallemend F, Aquino JB, et al. Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. Cell. 2009;139(2):366-379. https://doi.org/10.1016/j.cell.2009.07.049.

81. Nieto MA, Huang RYJJ, Jackson RAA, Thiery JPP. EMT: 2016. Cell. 2016;166(1):21-45. https://doi.org/10.1016/j.cell.2016.06.028.

82. Thiery JP, Acloque H, Huang RYJ, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139(5):871-890. https://doi.org/10.1016/j.cell.2009.11.007.

83. Hoek KS, Eichhoff OM, Schlegel NC, et al. In vivo switching of melanocytes between proliferative and invasive states. Cancer Res. 2008;68(3):650-656. https://doi.org/10.1158/0008-5472.CAN-07-2491.

84. Schlegel NC, von Planta A, Widmer DS, Dummer R, Christofori G. PI3K signalling is required for a TGFr-induced epithelial-mesenchymal-like transition (EMT-like) in human melanoma cells. Exp Dermatol. 2015;24:22-28. https://doi.org/10.1111/exd.12580.

85. Tuncer E, Calcada RR, Zingg D, et al. SMAD signaling promotes melanoma metastasis independently of phenotype switching. J Clin Investig. 2019;129(7):2702-2716. https://doi.org/10.1172/JCI94295.

86. Li FZ, Dhillon AS, Anderson RL, McArthur G, Ferrao PT. Phenotype switching human disease. Cancer Res. 2008;68(3):650-656. https://doi.org/10.1158/0008-5472.CAN-07-2491.

87. Bronner ME, Simões-costa M. The neural crest migrating into the 21st century Marianne. Curr Top Dev Biol. 2016;116:115-134. https://doi.org/10.1016/bs.ctdb.2015.12.003.

88. Vandamme N, Berx G. Melanoma cells revive an embryonic transcriptional network to dictate phenotypic heterogeneity. J Investig Dermatol. 2016;136(10):2049-2058. https://doi.org/10.1016/j.jid.2016.05.116.

89. Fang R, Zhang G, Guo Q, et al. Nodal promotes aggressive phenotypic transitions in human melanoma cell populations. Genome Res. 2018;28(9):1353-1363. https://doi.org/10.1101/gr.234062.117.

90. Tsoi J, Robert L, Paraiso K, et al. Multi-stage differentiation defines melanoma subtypes with differential vulnerability to drug-induced iron-dependent oxidative stress. Cancer Cell. 2018;33(5):890-904.e5. https://doi.org/10.1016/j.ccell.2018.03.017.

91. Tsol J, Robert L, Paraiso K, et al. Multi-stage differentiation defines melanoma subtypes with differential vulnerability to drug-induced iron-dependent oxidative stress. Cancer Cell. 2018;33(5):890-904.e5. https://doi.org/10.1016/j.ccell.2018.03.017.

92. Conway SJ, Kaartinen V. TGFr1 superfamily signaling in the neural crest lineage. Cell Adhes Migr. 2011;5(3):232-236. https://doi.org/10.4161/cam.5.3.15498.

93. John N, Cinelli P, Wegner M, Sommer L. Transforming growth factor β-mediated Sox10 suppression controls mesenchymal progenitor generation in neural crest stem cells. Stem Cells. 2011;29(1):698-699. https://doi.org/10.1002/stem.607.

94. Wurdak H, Ittner LM, Lang KS, et al. Inactivation of TGFr signaling in neural crest stem cells leads to multiple defects reminiscent of DiGeorge syndrome. Genes Dev. 2005;19(5):530-535. https://doi.org/10.1101/gad.317405.
111. Sáez-Ayala M, Montenegro MF, Sánchez-del-Campo L, et al. Directed phenotype switching as an effective antimelanoma strategy. *Cancer Cell*. 2013;24(1):105-19. https://doi.org/10.1016/j.ccr.2013.05.009.

112. Li J, Cao F, Yin HL, et al. Ferroptosis: past, present and future. *Cell Death Dis*. 2020;11(2):88. https://doi.org/10.1038/s41419-020-2298-2.

113. Mort RL, Jackson IJ, Elizabeth Patton E. The melanocyte lineage in development and disease. *Development (Cambridge)*. 2015;142(4):620-632. https://doi.org/10.1242/dev.106567.

114. Rebecca VW, Somasundaram R, Herlyn M. Pre-clinical modeling of cutaneous melanoma. *Nat Commun*. 2020;11(1):2858. https://doi.org/10.1038/s41467-020-15546-9.

115. McConnell AM, Mito JK, Ablain J, et al. Neural crest state activation in NRAS driven melanoma, but not in NRAS-driven melanocyte expansion. *Dev Biol*. 2019;449(2):107-114. https://doi.org/10.1016/j.ydbio.2018.05.026.

**How to cite this article:** Diener J, Sommer L. Reemergence of neural crest stem cell-like states in melanoma during disease progression and treatment. *STEM CELLS Transl Med*. 2021;10:522–533. [https://doi.org/10.1002/sctm.20-0351](https://doi.org/10.1002/sctm.20-0351)