Dynamic tumor heterogeneity in melanoma therapy: how do we address this in a novel model system?

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Targeted therapy of metastatic melanoma with MAPK pathway inhibitors holds great promise but suffers from rapid onset of resistance. The molecular mechanisms leading to drug resistance are discussed extensively in the literature [1]. Other possible contributing factors to drug sensitivity are tumor heterogeneity and differential biologic behavior, such as cell cycle progression, of melanoma cells in distinctive areas of the tumor [2].

Tumor heterogeneity

While dysregulated proliferation is a hallmark of cancer [3,4], not all tumor cells behave the same way: Solid cancers, including melanoma, are typically composed of irregular zones of actively proliferating and quiescent cells. Although the molecular mechanisms underlying aberrant cell cycle progression of cancer cells are well understood [5,6], not much is known about the regulation of the heterogeneous cell cycle dynamics of single cancer cells or groups of cancer cells within the complex tumor microenvironment. As different tumor subcompartments may have distinctive impact on metastasis

and drug resistance, deeper knowledge of this phenomenon will be critical for the development of novel therapy approaches. The phenomenon of tumor heterogeneity has been explained by a number of different, but not necessarily mutually exclusive, models. Both clonal evolution of cancer cells and the existence of cancer stem cells have been discussed as causes of tumor heterogeneity over the past decades [7,8]. While the molecular and/or epigenetic changes that result in phenotypic and functional differences between cells in these two models are characteristically irreversible [9], cancer cell plasticity as a third cause of tumor heterogeneity is a reversible and therefore dynamic process. For example, melanoma cells can reversibly shift between invasive and proliferative modes as described in the phenotype-switching model [10,11], or distinct melanoma cell subpopulations can temporarily reside in a slow-cycling mode [12]. In addition to these cell intrinsic factors regulating tumor cell proliferation and migration dynamics, extrinsic factors within the microenvironment such as nutrient and oxygen supply, availability

KEYWORDS

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- melanoma heterogeneity
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- tumor microenvironment

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of growth factors and interactions with the extracellular matrix affect the metabolic state of tumor cells and consequently their proliferative and/or invasive activity [2]. The fact that melanomas contain areas of actively cycling and areas of quiescent cells [13,14] is important, as cell cycle-arrested tumor subcompartments could escape the actions of anticancer drugs [15,16].

A novel approach for studying cell-cycle progression in a dynamic manner

Established methods for studying cell-cycle progression usually look either at entire cell populations (e.g., DNA content analysis by flow cytometry) or provide static snap shots of individual cells (e.g., Ki67 staining of tissue sections). A novel approach has been developed that labels key proteins of specific cell cycle phases in live cells, Cdt1 and Geminin. These proteins accumulate specifically in G1 and S/G2/M phases, respectively. The fluorescent ubiquitination-based cell cycle indicator (FUCCI) system incorporates fusion constructs of human Cdt1 and monomeric Kusabira Orange (mKO2-hCdt1) as well as of human Geminin and monomeric Azami Green (mAG-hGem) [17]. This system therefore allows us to follow individual cycling cells in real time whereby the nuclei of FUCCI-transduced cells transition from red (G1) to yellow (early S) and green (S/G2/M) fluorescence prior to cytokinesis, which is followed by a brief period of fluorescence negativity of the two daughter cells [17]. We have recently generated stable FUCCI-expressing clones of a number of human melanoma cell lines [2]. We have also previously developed a 3D melanoma spheroid model that more faithfully recapitulates the biology of melanomas *in vivo* as compared with 2D culture [18,19]. This model allows quantification of tumor viability, growth and invasion in untreated spheroids as well as in response to therapy and predicts the *in vivo* situation much better than 2D culture [19,20]. By time-lapse confocal imaging of FUCCI-expressing melanoma spheroids we showed that the initial distribution of green and red cells was random but that over time actively cycling cells (red, yellow and green) sequestered in a ring-like pattern at the spheroid periphery, while most cells in the center remained in G1 (red) [2]. The proliferating melanoma

cell subpopulation in the spheroid periphery correlates with the subpopulation expressing enhanced ERK activity [21]. We confirmed this phenomenon in human melanoma xenografts in mice, where there was a distinct distribution of clusters of cycling cells near the tumor's edge and near blood vessels contrasting with clusters of quiescent cells in more central areas of the tumors [2]. Hence, a likely explanation for the segregation of cycling melanoma cells within spheroids and tumors is the differential access to nutrients and oxygen, which is limited in the spheroid or tumor centers [15,19–20]. Moreover, re-exposure of inner cells to a more favorable environment revealed that G1 arrest of melanoma cells in areas of suboptimal nutrition and oxygen supply is a rapidly reversible phenomenon [2]. Together, these data support the model of microenvironment-driven dynamic heterogeneity in melanoma.

Future perspective

More research into the mechanisms underlying melanoma drug resistance is needed. Indeed, our model has already contributed to the finding that phenotypic plasticity as an early innate stress response causes acquired multidrug tolerance in melanoma [22]. Cutting edge imaging technology using the FUCCI-system will allow us to better understand the biology of dynamic heterogeneity, which is critical for the development of novel melanoma treatment strategies as drug sensitivity and resistance are closely linked to this phenomenon.

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References

- 1 Tonnessen CA, Haass NK. Melanoma: from tumor specific mutations to a new molecular taxonomy and innovative therapeutics. In: *Personalized Treatment Options in Dermatology*. Bieber T, Nestle F (Eds). Springer Berlin Heidelberg, Germany, 7–27 (2015).
- 2 Haass NK, Beaumont KA, Hill DS *et al*. Real-time cell cycle imaging during melanoma growth, invasion, and drug response. *Pigment Cell Melanoma Res.* 27(5), 764–776 (2014).
- 3 Hanahan D, Weinberg RA. Hallmarks of cancer. *Cell* 100(1), 57–70 (2000).
- 4 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 144(5), 646–674 (2011).
- 5 Pavey S, Spoerri L, Haass NK, Gabrielli B. DNA repair and cell cycle checkpoint defects as drivers and therapeutic targets in melanoma. *Pigment Cell Melanoma Res.* 26(6), 805–816 (2013).
- 6 Tsao H, Chin L, Garraway LA, Fisher DE. Melanoma: from mutations to medicine. *Genes Dev.* 26(11), 1131–1155 (2012).
- 7 Nowell PC. The clonal evolution of tumor cell populations. *Science* 194(4260), 23–28 (1976).
- 8 Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 414(6859), 105–111 (2001).
- 9 Shackleton M. Moving targets that drive cancer progression. *N. Engl. J. Med.* 363(9), 885–886 (2010).
- 10 Carreira S, Goodall J, Denat L *et al*. Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. *Genes Dev.* 20(24), 3426–3439 (2006).
- 11 Hoek KS, Eichhoff OM, Schlegel NC *et al*. *In vivo* switching of human melanoma cells between proliferative and invasive states. *Cancer Res.* 68(3), 650–656 (2008).
- 12 Roesch A, Fukunaga-Kalabis M, Schmidt EC *et al*. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell* 141(4), 583–594 (2010).
- 13 Brandner JM, Haass NK. Melanoma's connections to the tumour microenvironment. *Pathology* 45(5), 443–452 (2013).
- 14 Villanueva J, Herlyn M. Melanoma and the tumor microenvironment. *Curr. Oncol. Rep.* 10(5), 439–446 (2008).
- 15 Minchinton AI, Tannock IF. Drug penetration in solid tumours. *Nat. Rev. Cancer* 6(8), 583–592 (2006).
- 16 Haass NK, Schumacher U. Melanoma never says die. *Exp. Dermatol.* 23(7), 471–472 (2014).
- 17 Sakaue-Sawano A, Kurokawa H, Morimura T *et al*. Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *Cell* 132(3), 487–498 (2008).
- 18 Smalley KS, Lioni M, Noma K, Haass NK, Herlyn M. *In vitro* three-dimensional tumor microenvironment models for anticancer drug discovery. *Expert Opin. Drug Discov.* 3(1), 1–10 (2008).
- 19 Beaumont KA, Mohana-Kumaran N, Haass NK. Modeling melanoma *in vitro* and *in vivo*. *Healthcare* 2(1), 27–46 (2014).
- 20 Santiago-Walker A, Li L, Haass NK, Herlyn M. Melanocytes: from morphology to application. *Skin Pharmacol. Physiol.* 22(2), 114–121 (2009).
- 21 Haass NK, Sproesser K, Nguyen TK *et al*. The mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitor AZD6244 (ARRY-142886) induces growth arrest in melanoma cells and tumor regression when combined with docetaxel. *Clin. Cancer Res.* 14(1), 230–239 (2008).
- 22 Ravindran Menon D, Das S, Krepler C *et al*. A stress-induced early innate response causes multidrug tolerance in melanoma. *Oncogene* doi:10.1038/onc.2014.372 (2014) (Epub ahead of print).