European Journal of Cancer 59 (2016) 109–112

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

EJC Biennial Report

Phenotypic tumour cell plasticity as a resistance mechanism and therapeutic target in melanoma

Alexander Roesch [a,b,*], Annette Paschen [a,b], Jenny Landsberg [c], Iris Helfrich [a,b], Jürgen C. Becker [b,d], Dirk Schadendorf [a,b]

[a] *Department of Dermatology, Venereology, and Allergology, University Hospital Essen, University of Duisburg-Essen, 45122 Essen, Germany*
[b] *German Cancer Consortium (DKTK), Partner Site Essen/Düsseldorf, West German Cancer Center, University of Duisburg-Essen, 45122 Essen, Germany*
[c] *Department of Dermatology and Allergy, University of Bonn, 53127 Bonn, Germany*
[d] *Translational Skin Cancer Research, University Hospital Essen, University of Duisburg-Essen, 45147 Essen, Germany*

Received 17 February 2016; accepted 18 February 2016
Available online 26 March 2016

KEYWORDS
Melanoma;
Therapy resistance;
Tumour heterogeneity;
Tumour plasticity

Abstract Despite the recent success of MAPK and immune checkpoint inhibitors in advanced melanoma, intrinsic and acquired resistance mechanisms determine the efficacy of these therapeutic approaches. Therapy resistance in melanoma is not solely driven by genetic evolution, but also by epigenetically driven adaptive plasticity. Melanoma cells are shifting between different transcriptional programs, cell cycle states and differentiation phenotypes reflecting a highly dynamic potential to adapt to various exogenous stressors including immune attack or cancer therapies. This review will focus on the dynamic interconversion and overlap between different melanoma cell phenotypes in the context of therapy resistance and a dynamically changing multicellular microenvironment.

1. Introduction

Advances in the understanding of melanoma biology and immune regulation have led to the development of new drugs that can prolong overall survival in some patients with metastatic disease. However, despite the promising clinical data that have been reported for

* *Corresponding author:* Department of Dermatology, Venereology, and Allergology, University Hospital Essen, University of Duisburg-Essen, 45122 Essen, Germany.
E-mail address: alexander.roesch@uk-essen.de (A. Roesch).

http://dx.doi.org/10.1016/j.ejca.2016.02.023
0959-8049/

mitogen-activated protein kinase (MAPK) ($BRAF^{V600E}$ and MEK) inhibitors or CTLA4- and PD-1 checkpoint inhibitors in advanced melanoma, the majority of patients still die from recurring or persisting metastases. For example, the overall response rate for PD-1 inhibitors is ~40% indicating a high level of *a priori* resistance. Combined MAPK inhibition reaches overall response rates of up to ~70%, but acquire considerable resistance after ~6–9 months [1]. Modelling drug resistance in cancer is subject to ongoing research efforts and a multitude of genetic and epigenetic mechanisms have been discovered especially in melanoma [2]. Recent large-scale analyses incorporating transcriptome and methylome data even indicate a reciprocal connection to the immune evolution of melanoma cells during acquisition of MAPK resistance [3]. All together this points to an understanding of melanoma as a highly dynamic system, in which distinct cell phenotypes dynamically switch into each other depending on the current therapeutic and immune context.

2. Dynamic interconversion between different transcriptional programs, cell cycle states and differentiation phenotypes in melanoma

2.1. The phenotype switching model

Melanoma is a tumour with high heterogeneity and, as a reminiscence of its neuroectodermal origin, also high phenotypic plasticity allowing for rapid interconversion between different transcriptional profiles. Konieczkowski et al. have shown that RAF inhibitor-sensitive and -resistant $BRAF^{V600E}$-mutant melanoma cells can be discriminated by distinct RNA expression signatures. Sensitive melanomas displayed high activity of the master regulator of melanocytic development MITF and downstream differentiation markers like TYRP1, MLANA, and PMEL, whereas resistant melanomas had low MITF but high levels of inflammatory nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) signalling and the receptor tyrosine kinase (RTK) AXL [4]. Interestingly, NF-κB activation by exogenous tumour necrosis factor (TNF)α treatment reduced MITF expression in sensitive cell lines and induced a phenotypic transition towards resistance [4]. MITF and AXL are part of two opposing gene expression patterns, which have been previously identified by DNA microarrays of more than 80 melanoma cell lines irrespective of the genetic background (e.g. NRAS or BRAF mutated melanomas) [5]. Cells with the MITF-signature expressed melanocytic and neural crest lineage markers and were more proliferative, while cells with upregulation of inhibitors of the Wnt/β-catenin pathway such as Wnt5a or DKK1 were less proliferative, but highly invasive [5,6]. According to the 'MITF rheostat model' developed by Goding, these two phenotypes may reflect end-points of a broad spectrum of subpopulation identities. Melanoma cells may switch from a quiescent dedifferentiated ('stem-like') phenotype ($MITF^{low}$) to a proliferative phenotype ($MITF^{intermediate}$) and finally to a differentiated, again cell cycle arrested phenotype ($MITF^{high}$) [7,8].

2.2. Epithelial-to-mesenchymal transition

Melanoma phenotype switching and the involved signalling networks are strongly reminiscent of the concept of epithelial-to-mesenchymal transition (EMT), which has been classically linked to metastasis and therapy resistance in epithelial cancers [9]. Global gene expression profiling in mouse models and patients undergoing chemotherapy, immunotherapy, radiation or targeted therapies revealed an increase in mesenchymal markers in different tumour entities including melanoma [10–12]. In intrinsically MAPK inhibitor-resistant $BRAF^{V600E}$-mutant melanoma cells, JNK pathway activation has been lately identified as regulatory mechanism for the induction of EMT-characteristics [12]. However, the detailed impact of EMT on metastatic dissemination and drug resistance in melanoma is still not fully understood.

2.3. Cancer stemness as a dynamic trait of melanoma cell subpopulations

Cancer stem cells (CSC) are cells within a tumour that possess the capacity to self-renew and to cause all heterogeneous cell lineages that comprise the tumour [13]. Interestingly, invasive melanoma cells display features that are attributed to CSC, in particular in the context of drug resistance. For example, BRAFi-resistant melanoma cells develop a highly metastatic phenotype accompanied by upregulation of CSC-associated markers like CD271 or JARID1B [14,15]. JARID1B has been identified by us as a marker for slow-cycling melanoma cells with high potential to maintain tumour growth and to survive various cancer therapies. However, the $JARID1B^{high}$ phenotype does not follow a static tumour hierarchy (as postulated for classic CSC) and can be acquired also by cells from the regular tumour bulk, e.g. depending on microenvironmental changes like lowered oxygen levels [16]. Accordingly, others have found CD271 to be increased by hypoxia or low glucose levels [17]. These observations suggest that cancer stemness could be another facet of phenotypic tumour plasticity rather than an autonomous cancer model with strong overlap to EMT and the phenotype switching concept [2,16,18].

2.4. Influences of microenvironmental factors on phenotypic reprogramming

Hypoxia has been reported to upregulate the RTK ROR2 leading to activation of the non-canonical Wnt5a pathway [19]. Reciprocal to MITF, activation of Wnt5a supports the non-proliferating, highly invasive