The AhR-SRC axis as a therapeutic vulnerability in BRAFi-resistant melanoma

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Abstract

The nongenetic mechanisms required to control tumor phenotypic plasticity and shape drug-resistance remain unclear. We show here that the Aryl hydrocarbon Receptor (AhR) transcription factor directly regulates the gene expression program associated with the acquisition of resistance to BRAF inhibitor (BRAFi) in melanoma. In addition, we show in melanoma cells that canonical activation of AhR mediates the activation of the SRC pathway and promotes the acquisition of an invasive and aggressive resistant phenotype to front-line BRAFi treatment in melanoma. This nongenetic reprogramming identifies a clinically compatible approach to reverse BRAFi resistance in melanoma. Using a preclinical BRAFi-resistant PDX melanoma model, we demonstrate that SRC inhibition with dasatinib significantly re-sensitizes melanoma cells to BRAFi. Together we identify the AhR/SRC axis as a new therapeutic vulnerability to trigger resistance and warrant the introduction of SRC inhibitors during the course of the treatment in combination with front-line therapeutics to delay BRAFi resistance.

Keywords  BRAFi resistance; cell plasticity; expression; melanoma
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Introduction

Deciphering the genetic landscape of cancer led to a better understanding of tumor development, tumor annotation, and classification (Bailey et al, 2018). The identification of recurrent driver mutations underscored oncogenic addiction and designed new druggable targets revolutionizing patient care (Berger & Mardis, 2018). However, a major barrier to effective therapy is the capacity of cancer cells to resist. Melanoma represents a pioneering model to comprehend the multiple facets of resistance mechanisms.

The discovery of oncogenic BRAF mutations in about 50% of advanced melanomas has emerged as central, transforming melanoma therapy (Davies et al, 2002). The most common BRAF mutation consists of a T to A transition (T1799A), encoding a BRAF1500E oncogenic protein with constitutive kinase activity, leading to downstream MAPKinase signaling activation. Patient-tumors carrying such mutations are treated with BRAF inhibitors (BRAFi) namely vemurafenib (Bollag et al, 2010), dabrafenib (Hauschild et al, 2012), or encorafenib (Koelblinger et al, 2018), in combination with MEK inhibitors (MEKi) respectively cobimetinib (Larkin, 2014), trametinib (Salama & Kim, 2013; Robert et al, 2014; Daud et al, 2017), and binimetinib (Dummer et al, 2018; Shirley, 2018) to overcome BRAF paradoxical activation (Zhang et al, 2015) and maximize the therapeutic response. Under such front-line double blockade, patients show remarkable immediate responses. However, the response is transient, with median progression-free survival (PFS) of 15 months and a median overall survival up to 30 months (Michielin et al, 2020), followed by the development of resistance, leading to relapse and death (Dummer et al, 2018; Shirley, 2018).

Understanding the molecular mechanism of resistance to BRAFi/MEKi double blockade is critical to maximize clinical response. Unlike other oncogenic addicted tumors, namely EGFR driven lung cancer (NSCLC), where the appearance of secondary mutation in the target gene (EGFR) is a common mechanism of resistance to EGFR inhibitors (Kobayashi et al, 2005), no BRAF secondary mutation has been so far reported in BRAFi-resistant melanomas.

Resistance to MAPK inhibitors proceeds through different genetic route mainly mutation, amplification mechanisms, leading to reactivation of the MAPK pathway or MAPK-redundant signaling pathway such as activation of the PI3K/AKT pathway, along with the upregulation of tyrosine kinase receptors (TKRs; EGFR, IGF1R, PDGFR, AXL, etc.) (Arozarena & Wellbrock, 2017; Rossi et al, 2019; Czarnecka et al, 2020).

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In addition to these acquired genetic alterations, a new concept of resistance has emerged based on the capacity of melanoma cells to undergo transcriptomic reprogramming. Single cell transcriptomic analysis showed that the adaptive response to BRAFi is diverse, leading to the generation of a gradient of dedifferentiated cell states from melanocytic to neural crest state (Rambow et al., 2018; Tsoi et al., 2018). The plasticity of melanoma cells mediates a phenotype switching of the cells, which constitutes a robust escape route to therapy (Hoek et al., 2008; Kemper et al., 2014; Marin-Bejar et al., 2021). Under the control of the microenvironment or intrinsic cell factors, melanoma cells could switch from a proliferative to invasive state, acquiring resistance to targeted therapies. These phenotypic changes are mainly associated with a process of dedifferentiation similar to the epithelial-to-mesenchymal transition (EMT-like) that promotes metastatic spreading (Carreira et al., 2006; Hoek et al., 2008; Cheli et al., 2012; Verfaillie et al., 2015; Dilshat et al., 2021). Nonetheless, in some cases, melanoma cells still exhibit a differentiated state and are resistant to BRAFi (Tirosh et al., 2016; Rambow et al., 2018). Transcription factors, such as the master regulator of the melanocytic lineage, the microphthalmia-associated transcription factor (MITF) plays a critical and founding role in directing melanoma cell plasticity (Wellbrock & Marais, 2005; Müller et al., 2014; Noguchi et al., 2017; Goding & Arneither, 2019). While MITF\textsuperscript{high} state is associated with melanocyte differentiation and drives melanoma proliferation (Hoek et al., 2008; Rambow et al., 2018), the MITF\textsuperscript{low} (Müller et al., 2014) state is associated with drug resistance, supporting the notion of transcriptional balance.

We demonstrated that the Aryl hydrocarbon Receptor (AhR) transcription factor is constitutively activated in a subset of melanoma cells, promoting the dedifferentiation of melanoma cells and the expression of BRAFi-resistant genes (Corre et al., 2018). Using two complementary genome-wide CRISPR/Cas9 screens (CRISPR-a and CRISPR-i), we and others further underscored the role of AhR in the acquisition of BRAFi resistance (Gautron et al., 2021; Goh et al., 2021).

AhR is a ligand-activated transcription factor belonging to the family of the basic-helix–loop–helix (bHLH) Per-Arnt-Sim (PAS) transcription factor. In its inactive state, AhR is part of a cytosolic multi-protein complex that includes heat-shock protein 90, p23, AhR-interacting protein (AIP) and SRC (Enan & Matsumura, 1996; Cox & Miller, 2004; Nukaya et al., 2010). Upon ligand binding, AhR dissociates from its chaperone complex and translocates into the nucleus, where it interacts with its partner the AhR nuclear translocator (ARNT). AhR-ARNT nuclear dimers regulate the expression of target genes through recognition and binding to xenobiotic-responsive elements (XREs) located within the promoter of their target genes allowing cell specific gene expression programs. Remarkably, through ligand-binding interaction, AhR has the capacity to integrate environmental and cell-dependent signals (Denison et al., 2002) to shape and adapt the cell response, making AhR a very attractive candidate in regulating melanoma plasticity.

In addition, AhR activation has been shown to participate in the phosphorylation of the non-receptor tyrosine kinase SRC (Y416 residue) (Randi et al., 2008; Tomkiewicz et al., 2013; Fallahi-Sichani et al., 2017). SRC is known to be involved in many cellular functions, including the promotion of tumor-cell survival, motility, and invasion, through a rapid activation of focal adhesion kinase (FAK) contributing to cell migration and EMT (Nihal & Wood, 2016; Patel et al., 2016). However, the relation between AhR and SRC has not yet been explored in melanoma.

Herein, we aimed to delineate the role of AhR in orchestrating melanoma phenotypic switching during the acquisition of resistance through genomic and non-genomic routes. In particular, we pinpointed the crosstalk between AhR and SRC in reshaping cell fate and identify the AhR/SRC axis as a new therapeutic vulnerability for the treatment of BRAFi-resistant melanoma.

**Results**

**AhR controls acquisition of the invasive phenotype of melanoma**

AhR is markedly expressed in highly dedifferentiated, resistant, and invasive melanoma cells, mediating resistance to BRAFi (Corre et al., 2018). Accordingly, genetic depletion of AhR in BRAFi-resistant SKMel28 melanoma cells (SK28R) by CRISPR/Cas9 technology (AhR KO; Fig EV1A) significantly reduced their resistance to various BRAFi (vemurafenib, dabrafenib, and encorafenib; Fig 1A). We specifically evaluated the role of AhR in controlling cell migration by performing wound-healing assays of BRAFi-sensitive or resistant melanoma cells (called SK28S and SK28R, respectively) after genetic depletion of AhR (SK28S/R KO; Fig 1B) or chemical inhibition using its specific antagonist (CH-223191, 10 μM; Fig 1C). The loss or inhibition of the AhR significantly reduced migration capacity of melanoma cells (Fig 1B and C). Next, we analyzed the invasive properties of SK28S and SK28R melanoma cell lines using tumor-spheroid assays, which mimic the 3D architecture of melanoma. BRAFi-resistant cells were far more invasive than BRAFi-sensitive cells (Fig 1D) and the loss of the AhR transcription factor significantly reduced invasion of SK28R cells on a collagen matrix at 4 days (Fig 1D). Comparable results were obtained by chemical inhibition (CH-223191, 10 μM) of AhR in SK28 cells and no effect was observed in AhR KO cells (Fig 1E), underscoring the specificity of the CH-223191 and AhR function.

The role of AhR transcription factor in governing the resistance and invasive capacity of melanoma cells was further highlighted after increasing the endogenous expression of AhR by CRISPR/SAM technology in melanoma cells expressing low levels of AhR protein (501Mel) or after rescuing SK28R AhR KO cell lines with a constitutively activated form of AhR, (CA-AhR; McGuire et al., 2001). CRISPR/SAM stable expression of endogenous AhR in 501Mel cells was obtained using two different single-guide RNAs (sgRNA) targeting the AhR promoter region, with a subsequent increase in the capacity to mediate the expression of AhR (Fig 2A). Increased AhR expression reduced slightly BRAFi sensitivity (Fig 2B) and increased the invasive capacity of the 501Mel cells (Fig 2C) according to the ability of the sgRNA to induce AhR expression. Comparable results were obtained with the stable SK28R KO cell line expressing the constitutively active form of AhR (CA-AhR; Fig 2D). Significant increase of BRAFi resistance (Fig 2E) and invasive capacity were observed (Fig 2F). Thus, both AhR expression and its activation control the phenotype of melanoma cells and their sensitivity to BRAFi.

The sensitivity of melanoma cells to BRAFi has been associated with a highly differentiated cell state under the control of the MITF transcription factor (i.e., MITF\textsuperscript{high} or pigmentation signature) (Rose et al., 2016).
et al., 2016; Smith et al., 2016; Rambow et al., 2018). Conversely, we showed that AhR transcription factor participates in BRAFi resistance (Corre et al., 2018). To characterize the molecular role of AhR in such transcriptional reprogramming, we compared specific gene expression signatures (Invasion, Resistance, Proliferation, Melanocytic... corresponding to the median of gene expression of previously established gene-signatures associated with melanoma phenotype as described in Appendix Table S1). These comparisons were performed with the SK28S and SK28R melanoma cell lines before and after genetic depletion of AhR (SK28S/R KO) and its rescue with AhR constitutive active form (CA-AhR; from RNAseq data, GSE166617; Figs 2G, and EV1B and C). As we previously described and underscored here in Fig 2G, the β-signature (associated with BRAFi sensitivity; Corre et al., 2018) was highly represented in the proliferative, differentiated (Melanocytic and Transitory; Tsoi et al., 2018), and BRAFi-sensitive cell lines (SK28S). Conversely, the α-signature depicting canonical activation of AhR (Corre et al., 2018) was most prominent in dedifferentiated (neural crest-like and undifferentiated) BRAFi-resistant lines and co-occurred with the resistance signature (SK28R; Fig 2G). The absence of AhR expression (SK28R-KO) significantly decreased the expression of these gene-signatures (Fig 2G), while the re-expression of the constitutively active form of AhR (SK28R-KO CA-AhR) led to their overexpression (Fig 2G). Interestingly, these AhR associated signatures segregate BRAFi resistant melanoma cells from the sensitive ones (Cancer Cell Line Encyclopedia – CCLE RNA-seq data (Barretina et al., 2012)) and in Melanocytic-Transitory from Neural Crest like-Dedifferentiated melanoma cells (GSE80824 (Tsoi et al., 2018); Appendix Fig S1A) and in invasive melanoma cell lines (Appendix Fig S1B; Verfaillie et al., 2015). Besides the role of AhR in the regulation of resistance gene expression, we underscored its role in the acquisition of the dedifferentiated/invasive/mesenchymal phenotype. Overall, these results report that AhR mediates specific gene signature controlling the phenotypic switch of melanoma cells.

AhR regulates the expression of genes associated with BRAFi resistance, invasion, and dedifferentiation phenotypes of melanoma

To further decipher the direct role of AhR in the acquisition of the BRAFi-resistant associated phenotype we compared the previously established gene phenotype-signatures (Appendix Table S1) with RNAseq data from 501Mel cells exposed to BRAFi (Vem, 1 μM) or AhR ligand (TCDD, 10 nM) for 48 h (GSE104869 (Corre et al., 2018)), and with ChiP-Seq data identifying AhR target genes following exposure to TCDD (GSE90550 (Yang et al., 2018); Fig 3A and Appendix Fig S2A). This led to the selection of 216 genes predicted to be regulated by AhR (Appendix Fig S2A). Among these genes, 92 were significantly enriched (GSEA) in the sensitive/differentiated phenotype and 75 in the resistant/dedifferentiated one (Appendix Fig S2B and C, and Dataset EV1). The 50 most highly enriched genes between these two states (25 sensitive/differentiation genes (green) and 25 resistant/dedifferentiation genes (red), Dataset EV1 and Fig 3B), segregated sensitive and resistant SK28 cells (GSE166617; Fig 3B). AhR knockout (SK28R KO) and expression of its constitutively active form confirmed the involvement of AhR in the regulation of these resistant genes (in red, Fig 3B and C). Comparable results were obtained in 501 Mel cells overexpressing endogenous AhR (CRISPR/SAM; Fig EV1D). Finally, these 50 AhR-associated genes segregated BRAFi-resistant melanoma cells from sensitive ones (CCLE RNA-Seq data (Barretina et al., 2012)) and Melanocytic-Transitory melanoma cells from Neural Crest like-Dedifferentiated ones (GSE80824 (Tsoi et al., 2018); Fig 3B).

Interestingly, several of these AhR-associated genes have been involved in the aggressiveness of melanoma or other cancers (Appendix Table S2) and have been associated with a poor prognosis (ABCG2, COL1A1, COL6A1, COL6A2, TGFBI). CCL2, CRIM1, COL1A1, 6A1, 6A2 participate in cell migration, invasion, or EMT and ABCG2, ALDH1A1, NES are cancer stem-cell markers. Furthermore, AhR has been shown to directly regulate the expression of some of them (ABCG2, CCL2, STC2, etc.; Appendix Table S2), supporting the role of AhR in resistance.

We next explored the clinical relevance of this AhR-associated genes-signature by first examining melanoma samples from the TCGA cohort (Anaya, 2016). Among, the analyzed melanoma samples (n = 454), 17% of Patients strongly expressing AhR-associated resistance genes (red box) showed significantly lower overall survival than those highly expressing genes for sensitivity (blue box; Appendix Fig S3A). We investigated the expression of these genes in melanoma patients exposed to single drug-blockage (BRAFi) by classifying their melanoma biopsies during the course of medication and disease progression (baseline, early: DP1, intermediate: DP2, late: DP3; RNAseq dataset from Hugo et al (2015), GSE65185). Again, their expression level decreased at the beginning of the treatment (response to BRAFi phase: DP1) and slowly but significantly increased during the acquisition of BRAFi resistance (Fig 3D). Using, additional RNAseq data from melanoma cells lines (M229 and M397; GSE110054 (Tsoi et al., 2018)), we confirmed that the acquisition of BRAFi resistance correlates with a late increase in the expression of AhR-associated gene-signature (Appendix Fig S3B). The BRAFi/MEKi double blockade led to similar reprogramming of gene expression (Appendix Fig S3C).

Canonical activation of AhR triggers the SRC pathway to promote the BRAFi-resistant/invasive phenotype of melanoma

AhR is part of a cytosolic multiprotein complex with HSP90 and the SRC kinase (Enan & Matsumura, 1996; Rey-Barrosso et al, 2013) (Fig 4A). We investigated the potential cross-regulation between AhR and SRC signaling. We performed co-immunoprecipitation experiments to determine whether AhR and SRC are present in the same protein complex in melanoma cells (SKMel28). AhR was detected in the SRC immunoprecipitate (Fig 4A) and this interaction was confirmed by proximity ligation assay (Fig EV2A). We next tested whether AhR controls the activation of the SRC kinase in the context of BRAFi resistance. To this end, we examined their protein levels and the phosphorylated form (AhR, SRC, P-SRC, etc.) in four melanoma cell lines with increasing levels of BRAFi resistance (Figs 4B and EV2B). Concomitant to BRAFi resistance, we observed increased AhR protein levels and increased activation of SRC after phosphorylation on residue Tyr416 (Y416). Activation of FAK (phosphorylation on tyrosine 576/577) followed SRC phosphorylation (Figs 4B and EV2B). We next examined SRC and FAK protein levels and phosphorylation state in SK28R cells expressing or lacking AhR (Figs 4C and EV2C). While constitutive loss of AhR did not significantly induce SRC or FAK activation, rescue experiments with AhR
Figure 1.
Anais Paris et al

EMBO Molecular Medicine

5 of 19

constitutive active form (CA-AhR) led to a significant phosphorylation of SRC and FAK (Figs 4C and EV2C). Over-night exposure of SK28R cells to AhR canonical ligands (BaP, indirubin, ITE, TCDD, and FICZ), induced a massive phosphorylation of SRC on the Tyr416 residue. Concomitantly, AhR level diminished, underscoring its activation and subsequent degradation (Fig 4D). The level of SRC phosphorylation (P-Y416) increased with increasing doses of the AhR ligand ITE, (Fig 4E) within only a few minutes after activation (Fig EV2D and E). This resulted in the up-regulation of the expression of SRC-related genes, such as THBS1 and MMP1 (Said et al, 2017; Fig EV2F).

Sustained canonical activation of AhR mediates BRAFi resistance and the activation of the SRC/FAK pathway. To delineate the contribution of AhR-SRC axis in resistance acquisition, we established the gene expression profile (RNA-seq) of SK28R cells exposed to AhR agonist ITE (5 μM, 24 h) or to dasatinib (Das), a specific inhibitor of SRC (1 μM, 24 h). Comparative analysis of differentially expressed genes in SK28R cells exposed to ITE (10 μM for 24 h; Appendix Fig S4A) or Das (1 μM for 24 h; Appendix Fig S4B) allowed the identification of a significant number of genes with inversely correlated expression patterns (Appendix Fig S4C and D). Functional annotation (Dataset EV3) identified differentially expressed genes in focal adhesion (PDGFC, THBS1, ITGA3...), PI3K-Akt signaling, ECM-receptor interaction; pathways previously shown to be associated with BRAFI resistance and invasion (Ruffini et al, 2013; Vizkeleti et al, 2017; Zhang et al, 2020).

GSEA in different melanoma cell lines from the CCLE (Barrettina et al, 2012), and Tsoi et al datasets (GSE80824 (Tsoi et al, 2018)) using oncogenic signature gene sets (https://www.gsea-msigdb.org/gsea/msigdb/gene sets.jsp?collection=C6) underscored that the activation of several pathways (EGFR, YAP, KRAS, TCFl, Integrin, etc.) correlates with the mechanisms of BRAFI resistance associated with the dedifferentiation process (Appendix Fig S5A and Datasets EV1 and EV2). They include the SRC, FAK, and focal-adhesion pathways. Such induction was also observed in both BRAFi-resistant melanoma cell lines (Fig 4F and Appendix Fig S5B; Tsoi et al, 2018) and patients (Appendix Fig S5C; RNAseq dataset from Hugo et al (2015), GSE65185). RNAseq performed on SK28 cells before and after knockout of AhR or after canonical activation of the transcription factor confirmed the role of the AhR to induce the expression of genes (in bold) belonging to the integrin, SRC, FAK, and focal-adhesion pathways (Fig 4G and Appendix Fig S6A and B). Together this underlines AhR-induced genomic and non-genomic reprogramming of melanoma cells.

Inhibition of SRC sensitizes melanoma cells to BRAFi treatment and disrupts the acquisition of an invasive phenotype

Having pinpointed the cellular role of AhR in directing BRAFi resistance, we explored new therapeutic opportunities. Using the CellMiner database (https://discover.nci.nih.gov/cellminerdb), we correlated the therapeutic efficacy of a library of 300 chemical compounds (IC50) according to AhR mRNA levels (Z-score) in various cancer cell lines (lung, brain, CNS, breast, skin; Appendix Fig S7). Volcano plots showed a significant correlation (P < 0.001) for several drugs and scored the SRC inhibitor (dasatinib, Das) to be potentially effective in the context of BRAFi resistance (Appendix Fig S7). Correlative analysis (Das efficacy/gene expression) further showed that the SRCI Das was more effective in cell lines with a high level of AhR mRNA and strongly expressing genes mediating resistance, invasion, and melanoma dedifferentiation (Appendix Fig S8A and Table S3). The expressions of genes associated with AhR and SRC/FAK signatures were strongly correlated with the efficacy of Das (Appendix Fig S8B and C).

The two ATP-competitive protein tyrosine kinase inhibitors of SRC (bosutinib (Bos), dasatinib (Das)) were very effective resulting in the complete loss of the phospho-activated SRC form (P-Y416) in SK28R melanoma cells (WT, KO-AhR, CA-AhR; Fig EV3A). Concomitantly, we observed a decrease in the expression of SRC related genes (THBS1, MMP1; Fig EV3B). Treatment of SK28R melanoma cells with increasing doses of SRC inhibitors (Bos or Das) at different doses (10–500 nM) in combination with increasing doses of Vem significantly increased BRAFi sensitivity (Fig 5A). To analyze the effect of SCRi on cell viability, SK28R melanoma cells were treated alone with increasing doses of SRC inhibitors (Bos or Das, up to 0.5 μM) or in combination with BRAFi. Alone SCRi affected cell viability when used at relatively high concentration (> 0.125 μM for Bos and > 0.031 μM for Das). In contrast, when...
used in combination with BRAFi, the effect on cell viability was observed at low doses of SRCi (< 0.015 μM for Bos and < 0.0078 μM for Das; Fig 5A). Since it has been shown that SRCi alone had poor effect on BRAFi-sensitive melanoma cells, together it suggests that SRCi may resensitize resistant-melanoma cells to BRAFi.

Figure 2.
Das also sensitized other resistant melanoma cell lines (M229R and M238R), to BRAFi (Fig EV3C). In addition to their roles in sensitizing melanoma cells to BRAFi, the SRC inhibitors Bos and Das, even at low doses (1 μM), prevented the invasive capacity of wild-type AhR melanoma cells (red) in three-dimensional spheroid assays (Fig 5B). Das was also able to reduce the low-invasive capacity of KO- AhR melanoma cells (green; Fig 5B). Remarkably, Das blocked invasion induced by AhR activation (ITE, TCDD; Fig 5C) without affecting AhR protein level (Fig 5D) but by significantly reducing AhR transcriptional activity, alone or after AhR activation by TCDD measured by Luciferase assay (Fig 5E). To further support this identified AhR/SRC cross-regulation, we performed RNAseq on SK28R cells before or after treatment with Bos or Das and characterized the effect of SRC inhibitors on gene-reprogramming signature and AhR-target genes. Both inhibitors significantly decreased the expression of genes associated with SRC, FAK, focal adhesion and invasive/dedifferentiation signatures and AhR-targets (Fig 5F).

This *in vitro* evidence prompted us to examine the clinical relevance of using SRC inhibitors to resensitize BRAFi-resistant tumors to BRAFi. To this end, we used the Mel006R BRAFi-resistant patient-derived xenograft (PDX) mice model. The PDX line MEL006R is a BRAFV600E mutant cutaneous melanoma derived from MEL006 PDX lesions at relapse (Vendramin et al, 2021) upon acquisition of resistance to BRAFi/MEKi (Dabrafenib and Trametinib). Once tumors reached 200 mm³, grafted mice were treated with different treatment regimens: single-drug regimens (BRAFi or SRCi alone) or sequential administration (SRCi alone during the early growth phase of the tumor (16 days) followed by a BRAFi/SRCi double blockade). The growth of the tumor was monitored each 2 days until the tumor reached 1,500 mm³ (Fig 6A). As anticipated, BRAFi alone was largely ineffective in controlling the tumor growth of this BRAFi-resistant PDX (Figs 6B and EV4A). On the contrary, SRCi alone induced significant control of the tumor growth and the mice under SRCi survived significantly longer than the ones treated with BRAFi alone (Figs 6B and EV4A). Together this reinforces the role of SRC activation as an escape route to BRAFi. Remarkably, introducing BRAFi, 16 days post treatment with SRCi (Fig 6A) or by combining BRAFi and dasatinib from the start of treatment (Appendix Fig S9) significantly diminished the tumor growth rate. As a consequence, those mice survived significantly longer, with an overall survival rate almost doubled compared to mice treated with BRAFi alone. In conclusion, dasatinib after specific inhibition of SRC phosphorylation (Fig EV4B) significantly resensitized resistant tumors to BRAFi treatment and significantly increased the overall survival (Fig 6B–D). These results emphasize the therapeutic interest of SRCi for BRAFi-resistant patients.

**Discussion**

The acquisition of resistance to targeted therapy can be in part mediated by transcriptional reprogramming, eliciting a phenotypic...
Figure 3. 

A. Signature genes

Induction by TCDD (Corre et al. 2018)

ChIP AhR (Yang et al. 2017)

Induction by BRAFi (Corre et al. 2018)

AhR target genes involved in BRAFi resistance and dedifferentiation (n=216)

B. Heatmaps showing gene expression profiles in SKMel28, CCLE, and TSOI et al. 2018.

C. Heatmap showing gene expression in SK28R, SK28R KO, and SK28R KO CA-AHR.

D. Heatmap showing BRAFi treatment effects on gene expression.

Hugo et al. 2015
Figure 4. Canonical activation of AhR induces the activation of SRC/FAK associated with an increased activation of the focal adhesion pathway in BRAFi-resistant and dedifferentiated melanoma cell lines.

A At the basal level, AhR is located at the membrane layer in a protein complex that includes HSP90, p23, XAP, and SRC. Immunoprecipitation of SRC was performed with specific antibodies and SRC/AhR interaction analyzed by western blotting.

B Protein levels of the AhR, p-SRC (Y416), SRC, p-FAK (Y576/577), and FAK in the four different melanoma cell lines (n = 3). The level of BRAFi resistance corresponds to our measure of IC50 (Vemurafenib) for the different cell lines: 501Mel (0.23 μM), SKMel28 (0.29 μM), M229 (0.89 μM), and M238 (2.16 μM). These cell lines correspond to the sensitive parental cells.

C Protein levels of the AhR, p-SRC (Y416), SRC, p-FAK (Y576/577), and FAK in the SK28R cell line KO or not for AhR by CRISPR/Cas9 or after rescue with the activated-form of the AhR (CA-AhR).

D Protein levels of AhR, p-SRC (Y416), and SRC, in the SK28R cell line after treatment with different AhR ligands for 24 h (5 μM BaP, 5 μM indirubin, 5 μM ITE, 10 nM TCDD, and 5 μM FICZ).

E Protein levels of AhR, p-SRC (Y416), and SRC, in the SK28R cell line after 24 h of treatment with increasing doses of ITE.

F Expression heatmap for SRC, FAK, focal-adhesion, and integrin signatures in four different BRAFi-sensitive and resistant melanoma cell lines.

G Expression heatmap for SRC, FAK, focal-adhesion, and integrin signatures in the SK28R cell line KO or not for AhR by CRISPR/Cas9 or after rescue with the activated-form of the AhR (CA-AhR). The scale corresponds to the Z scores.

Source data are available online for this figure.
Figure 5.
switch toward distinct drug-tolerant transcriptional states of melanoma cells (Rambow et al., 2018; Tsoi et al., 2018).

Here, we identified a dual role of AhR transcription factor in the control of cell plasticity and phenotypic change during the acquisition of BRAFi resistance. First, high level and activity of AhR mediates the invasive/dedifferentiated phenotype of melanoma through the direct regulation of the expression of many genes involved in invasion (COL1A1, COL6A1, COL6A2, CYR61, STC2...). (Hoek et al., 2008; Verfaillie et al., 2015) and differentiation phenotypes (CCL2, NTN, NUA2K, SOX9, ABCG2...) (Rambow et al., 2018; Tsoi et al., 2018). (Appendix Table S2). Interestingly, the phenotype of melanoma cells lacking Mtt transcription factor was similar to those observed while AhR is highly expressed and activated (Dilshat et al., 2021), allowing to consider a new balance between these two transcription factors for the control of melanoma plasticity.

Second, sustained activation of AhR mediates the activation of the SRC pathway following phosphorylation of the Tyr416 (Y416). Together, AhR-dependent transcriptional reprogramming and SRC activation triggers the cell plasticity of BRAFi-resistant melanoma. The identification of an AhR/SRC regulation node fully supports the importance of non-genomic cell reprogramming. It also provides a strong rationale for the understanding of the role of the SRC-family in BRAFi treatment (Girotti et al., 2015; Close et al., 2020; Krayem et al., 2020) and allows to delineate the pathway that mediates the activation of SRC and elevated integrin/FAK observed in melanoma (Hirata et al., 2015).

Indeed, the crucial role of SRC in many aspects of tumor development including migration, invasion and survival has warranted the use of SRC inhibitors to disrupt these effects in several cancer types (Roskoski, 2015). In this respect, SRC inhibitors have been tested in melanoma. However, the anti-proliferative effect of SRC inhibitors alone, on melanoma cells, was minor to no effect. Importantly cytototoxicity was mainly observed in cells that did not carry BRAF onco-mutation (Eustace et al., 2008; Halaban et al., 2019). In accordance, clinical studies using SRCi as a single agent showed only minimal therapeutic activity in stage III/IV chemotherapy-naive unresectable melanoma (Kluger et al., 2011). These results contrast with those supporting the use of SRC inhibitors in resistance settings in line with the upregulation of members of the SRC-family kinases (Girotti et al., 2013) and downstream SRC-dependent effectors such as MCF2 and VAV1, two DBL family members identified through a genetic screen as candidate drug resistance in melanoma cells (Feddersen et al., 2019). SRC inhibitors were also reported to promote a differentiated state through the upregulation of Mtt expression and downstream melanocytic markers (TYR, TRP1...) via the MAPK and CREB pathways (Ku et al., 2019). This gives some hints of how SRC may participate in melanoma cell reprogramming. The identification herein of the AhR/SRC activation loop in BRAFi-resistant melanoma gives rationale to these studies filling an important gap to understand cell plasticity and propose innovative therapeutic regimens.

We previously showed in a preclinical PDX melanoma mice model that antagonizing AhR delayed the emergence of resistant cells (Corre et al., 2018). Here, using a BRAFi-resistant PDX melanoma model, we demonstrated that SRC inhibition (dasatinib) significantly controlled tumor growth and remarkably re-sensitize melanoma cells to BRAFi (dabrafenib), doubling the overall survival rate compared to BRAFi alone. This allows us to envision new therapeutic settings using SRC inhibitors to resensitize tumor cells to BRAFi and to improve therapeutic benefits with delayed relapses. The time to introduce SRCi could be determined by monitoring the presence of circulating tumor DNA (ctDNA) in liquid biopsies as an early marker of tumor progression (Calapre et al., 2017). The detection of the BRAFV600E mutation could serve as the starting point to initiate co-treatment with SRCi.

Our results also underscored that AhR-dependent activation of SRC in BRAFi-resistant cells leads to the activation of FAK kinase after phosphorylation. Marin-Bejar et al., 2021 have recently shown that gains activity of FAK signaling is associated with the emergence of neural crest stem cell (NCSC) subpopulation in BRAFi/MEKi drug-tolerant cells known as minimal residual disease (MRD). This activation of FAK in melanoma cells is driven in part by a “paradoxical” activation of melanoma-associated fibroblasts and the induction of β1/FAK/SRC signaling (Hirata et al., 2015) but also after activation of GFRA2/GDNF expression and AKT activation (Marin-Bejar et al., 2021). Interestingly, FAK-inhibitors strongly decreased the emergence of the NCSCs in MRD lesions, and drastically delayed the onset of resistance to RAF/MEK inhibitors in preclinical PDX models. They also proposed to test combinations of both FAK and SRC inhibitors, such as dasatinib, as a more effective strategy to suppress the emergence of the NCSC population at MRD (Marin-Bejar et al., 2021).

In parallel, we underscored that AhR-dependent activation of SRC mediates the activation of the epidermal growth factor receptor
Figure 6.

A. PDX = MM006R

Day 0

200mm³

Day 16

Day X

1500mm³

- Vehicle
- BRAFi (Dabrafenib)
- SRCi (Dasatinib)
- SRCi (Dasatinib) (a) and then combo (BRAFi+SRCi) (b)

B. Tumour Volume (mm³)

Days

Vehicle
Dab (BRAFi)
Das (SRCi)
Das before combo

C. Comparison of Survival Curves

Log-rank (Mantel-Cox) test
Chi² = 73.61
P value < 0.0001

D. Days to reach endpoint size

Days

Median

18.5
25.5
38.5
47

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Biscardi contribute to an aggressive phenotype in multiple human tumors
tance and EMT transition (Gross,2019). In addition, EGFR activation after phos-
colon and lung cancer cells (Xie, 2012; Ye et al, 2018) and to contribute to an aggressive phenotype in multiple human tumors
(Biscardi et al, 1999). Accordingly, BRAFi resistance commonly corre-
relates with a high level of EGFR expression and a poor prognosis
(Luebker & Koepsell, 2019). In addition, EGFR activation after phos-
phorylation has been shown to be more highly associated with resis-
tivity than group treated with BRAFi alone (Dabrafenib) P < 0.05 *, P < 0.01 **.
C Kaplan–Meier survival curve for MEL006R mice treated with the different drugs. Comparison of survival curves have been performed using the nonparametric Log-
counteract resistance.

(EGFR). Indeed, we showed that activation of SRC leads to the reactiv-
ation of the EGFR after its phosphorylation (Y845) in BRAFi-
resistant melanoma cell lines (Fig EV5A–C). Such SRC-AhR cross talk has been previously described to mediate EGFR phosphorylation in
colon and lung cancer cells (Xie et al, 2012; Ye et al, 2018) and to contribute to an aggressive phenotype in multiple human tumors
(Biscardi et al, 1999). Accordingly, BRAFi resistance commonly corre-

Materials and Methods

Cell culture and reagents

Human melanoma cell lines (SK28, 501Mel, M229, and M238) were grown in humidified air (37°C, 5% CO2) in RPMI-1640 medium
(Thermo Fisher Scientific, Invitrogen, Waltham, MA, USA) supple-
mented with 10% fetal bovine serum (Eurobio, Les Ulis, France) and 1% penicillin–streptomycin antibiotics (Thermo Fisher Scientific).
SK28 (S + R) cells were obtained from J.C Marine at the VIB Center for Cancer Biology, VIB, Leuven, Belgium. M229 cells were obtained
from Graeber’s lab at the UCLA Molecular Biology Institute, Los Angeles, CA, USA. 501Mel cells (S) were obtained from the ATCC and 501Mel BRAFi-resistant cells (R) were obtained after 3 months of treatment with Vem (1 μM every 2 days). No difference of prolifer-
ation has been observed between resistant cells and parental ones.

Melanoma cells were grown in the absence of BRAFi treatment but
challenged every 2 weeks with BRAFi at the IC50 dose of the sensi-
tive corresponding cells to maintain a selective pressure. HEK 293T cells were obtained from the ATCC and grown in humidified air (37°C, 5% CO2) in DMEM medium (Thermo Fisher Scientific). All
cell lines were routinely tested for mycoplasma contamination.

Reagents

- **AhR ligands:** 2,3,7,8-tetrachlorodibenzo-p-dioxine (TCDD; Sigma
  Aldrich, St Louis, MO, USA, 48599), 2-(1’H-indole-3-carbonyl)-
thiazole-4-carboxylic acid methyl ester (ITE; Medchem Express,
  Monmouth Junction, NJ, USA HY-19317), benzo-a-pyrene (BaP;
  Sigma Aldrich, B1760), indirubin (Selleckchem, Houston, TX,
  USA, S2386), FICZ (6-formylindolo[3,2-b]carbazole; Medchem
  Express, HY-12451), and CH-223191 (Selleckchem, S7711).
- **BRAF inhibitors:** vemurafenib (Vem, PLX4032; Selleckchem,
  S1267), dabrafenib (Dab, GSK2118436; Selleckchem, S2807), and
  encorafenib (LGX818; Selleckchem, S7108).
- **SRC inhibitors:** dasatinib (Selleckchem S1021) and bosutinib (SKI-
  606; Selleckchem, S1014).
- **EGFR inhibitors:** gefitinib (Selleckchem S1025) and erlotinib (Sel-
  leckchem S7786).
- **Hsp90 inhibitor:** NVP-HSP990 (Selleckchem S7097).
- **DMSO** – Sigma-Aldrich (D8418).

CRISPR/Cas9 experiments

The AhR knockout was performed using CRISPR/Cas9 methodology.
The guide sequence targeting AhR (Sigma-Genosys, St Louis, MO, USA) was cloned into the GeneArt CRISPR Nuclease vector according to the manufacturer’s instructions (Life Technologies, Saint-Aubin, France). Next, 501Mel or SK28 cells were transfectioned with the vectors and the cells seeded 2 days later in 96-well plates at 0.5 cells/well for single-cell clonal expansion. The clones of interest were validated by DNA-sequencing, western blot analysis, and RT-qPCR.

CRISPR-SAM experiments

Lentiviral infections were used to obtain stable cell lines. Lentiviral production was performed as recommended (http://tronolab.epfl.
ch) using HEK 293T cells, psPAX2 (Addgene, Cambridge, MA, USA
#12260) and pVSV-G (Addgene, #14888) plasmids, and the required
vectors. Infections were performed overnight. To generate 501Mel

cells individually overexpressing AhR, 501Mel cells were first transduced to stably express dCAS-VP64 (Addgene, #61425) and MS2-P65-HSF1 (Addgene, #61426) before transduction with specific AhR sgRNAs (from Supplementary Table S of Gautron et al., 2021). Infected cells were selected using zeocin (600 μg/ml, 5 days). Lentivirus was manipulated in the biosafety level 3 containment laboratory core facility of the Biology and Health Federative Research Structure of Rennes (Biosit).

Overexpression of constitutively active form of AhR

The open reading frame for CA-AhR (McGuire et al., 2001) was cloned into pLL3.7 using Gblocks (IDT DNA, Coralville, IA, USA) and Gibson Assembly® Master Mix following the manufacturer’s recommendations (NEB, Ipswich, MA, USA). Lentiviral infection was used to obtain stable cell lines. Lentiviral production was performed as recommended (http://tronolab.epfl.ch) using HEK 293T cells, pVSV-G (Addgene #14888), pRSV-Rev (Addgene #12253), pMDLg/pRRE (Addgene #12251), and a target vector. Infections were performed overnight. To generate SK28 cells overexpressing constitutive AhR, cells were infected to stably express the pLL3.7-AhR-CA vector (pLL3.7 backbone, Addgene #11795). Infected cells were selected twice by fluorescence-activated cell sorting using GFP detection.

Cell density evaluation

Cell density was assessed using a methylene blue colorimetric assay. Briefly, cells were fixed for at least 30 min in 95% ethanol. Following ethanol removal, the fixed cells were dried and stained for 30 min with 1% methylene blue dye in borate buffer. After four washes with tap water, 100 μl 0.1 N HCl was added to each well. Plates were then analyzed with a spectrophotometer at 620 nm.

Wound healing migration assay

Briefly, cells were grown until confluent in 2-well silicone inserts (Ibidi®, Germany) placed in 12-well tissue culture dishes. The cell culture inserts were removed after 1 day. Afterward, the plates were washed with PBS and incubated at 37°C in fresh RPMI-1640 medium (Gibco BRL, Invitrogen, Paisley, UK) supplemented with 10% fetal bovine serum (Eurobio) and 1% penicillin-streptomycin antibiotics (Gibco, Invitrogen), either naive or in the presence of vehicle (DMSO) or CH-223191 (5 μM). The wound was photographed with a Leica inverted microscope at 5× magnification using an Axio Vert.A1 inverted microscope (Carl Zeiss) at 5× magnification. Invasion capacity was evaluated by determining the ratio between the maximum and initial diameter of the spheroid.

RNA extraction and RT-qPCR expression

RNA extraction & RT-qPCR expression was performed as previously described (Corre et al., 2018). The sequences of the primers used for the RT-qPCR experiments are available in Appendix Table S4.

Western blotting

Harvested cells were solubilized as previously described. Protein samples were denatured at 95°C, resolved by SDS-PAGE, and transferred onto Hybond™-C Extra nitrocellulose membranes (Amersham Biosciences, Bucks, UK). Membranes were probed with the appropriate antibodies Appendix Table S5 and the signals detected using a Fujifilm LAS-3000 Imager (Fuji Photo Film, Tokyo, Japan). Primary antibody information is available in Appendix Table S5. Horseradish-peroxidase-conjugated secondary antibodies were purchased from Jackson ImmunoResearch (Suffolk, UK) and used at a dilution of 1:10,000.

Immunoprecipitation

SKMel28-resistant cells were collected and lysed with cell lysis buffer (20 mM Tris–HCl, pH 8, 150 mM NaCl, 0.5 M EDTA) for 30 min on ice. The supernatant was incubated with 40 μl of protein G magnetic beads and 10 μl of SRC antibody (2108, Cell Signaling) or of normal rabbit IgG (sc2027, Santa Cruz Biotechnology) overnight at 4°C under rotation. Beads were washed with lysis buffer and eluted with 30 μl of Laemmli. Immunoprecipitates were analyzed by western blotting with appropriate antibody.

Luciferase activity

HaCaT keratinocytes (2.10⁵) were cultured in 12-well plates and transfected with the pGL3-XRE3-Luc construct carrying firefly luciferase. Transient transfection of cells was performed as previously described (Corre et al., 2018). After a 24-h period, cells were exposed to TCDD (10 nM) in the presence of increasing concentration of dasatinib (0.5–5 μM) overnight. Luciferase assays were then performed using a Promega kit according to the manufacturer’s instructions. Data are expressed in arbitrary units relative to the value of luciferase activity levels found in DMSO-exposed cells, arbitrarily set to 1 arbitrary unit (a.u.). Firefly luciferase activity was normalized to protein content using the Bicinchoninic Acid Kit from Sigma-Aldrich® and measured using a luminometer CLARIOStar (BMG Labtech).

Proximity ligation assay

The proximity ligation assay was used to visualize AhR/SRC complexes in SK28 cells. The cells, grown on glass coverslips, were fixed
with 4% PFA in 0.1 M phosphate buffer (15735-60S, Electron Microscopy Sciences) for 15 min at RT and PLA performed using the Duolink® in Situ detection Reagent Orange (DUO92007), Duolink® in Situ PLA® Probe Anti-Mouse PLUS (DUO92001), and Duolink® in Situ PLA® Probe Anti-Rabbit MINUS (DUO92005). SIGMA kits according to the manufacturer’s protocol. After blocking, the reaction was performed with the primary antibodies: mouse anti-AhR (C20, 1/100) and rabbit anti-SRC (1C12, 1/100). Following the ligation and amplification steps, the coverslips were immobilized on microscopic slides using mounting medium containing DAPI. The ligation step was omitted in the control. Imaging analysis was carried out using a delta vision system (Applied Precision). The number of foci was quantified for at least 30 cells.

**Patient-derived xenografts**

In collaboration with TRACE and after approval by the University Hospital KU Leuven Medical Ethical Committee (S54185) and written informed consent from the patient, PDX model MEL006R (BRAFi resistant) was established from an in-transit metastasis resected as part of standard-of-care melanoma treatment at the University Hospital KU Leuven. The procedures involving mice were performed in accordance with the guidelines of the IACUC and KU Leuven and carried out within the context of approved project applications P147/2012, P038/2015 and P098/2015. Fresh tumor tissue was collected in transport medium (RPMI1640 medium supplemented with penicillin/streptomycin and amphotericin B). Tumor tissue samples were fixed in 4% PFA in 0.1 M phosphate buffer (15735-60S, Electron Microscopy Sciences) for 15 min at RT and PLA performed using the Duolink® in Situ detection Reagent Orange (DUO92007), Duolink® in Situ PLA® Probe Anti-Mouse PLUS (DUO92001), and Duolink® in Situ PLA® Probe Anti-Rabbit MINUS (DUO92005). SIGMA kits according to the manufacturer’s protocol. After blocking, the reaction was performed with the primary antibodies: mouse anti-AhR (C20, 1/100) and rabbit anti-SRC (1C12, 1/100). Following the ligation and amplification steps, the coverslips were immobilized on microscopic slides using mounting medium containing DAPI. The ligation step was omitted in the control. Imaging analysis was carried out using a delta vision system (Applied Precision). The number of foci was quantified for at least 30 cells.

**Pharmacologic treatment of mice**

Mice with tumors reaching 200–300 mm³ were treated via daily oral gavage. Dabrafenib (Biorbyt) and/or dasatinib (Selleckchem) were dissolved in DMSO at a concentration of 30 mg/ml respectively, aliquoted and stored at −80°C. Each day a new aliquot was diluted 1:10 with phosphate-buffered saline and mice were treated with a dose of 30 mg/kg for dabrafenib alone, with dasatinib alone (30 mg/kg) or with the combination dabrafenib + dasatinib (30 mg/kg each) after a pretreatment with dasatinib for 16 days. Tumor volume was monitored with a caliper and calculated using the following formula: \( V = \frac{4}{3} \pi r^3 \) where \( r \) is the tumor radius. The endpoint of the experiment corresponds when tumor volume reaches 1500 mm³ according to ethical statements.

**RNA-Seq**

Total RNA was extracted from BRAFi-sensitive or resistant SK28, Mel501, and M229, cells before and after knockout out of AhR using the NucleoSpin RNA kit (Macherey Nagel, Düren, Germany). A complementary DNA library was prepared and sequencing performed according to the Illumina standard protocol by Beijing Novel Bioinformatics Co., Ltd. (https://en.novogene.com/). RNAseq was performed in collaboration with Novogene (Beijing, China). Libraries were generated from 500 ng total RNA using a Truseq Stranded mRNA kit (Illumina). The concentration of the library was first determined using a Qubit2.0 fluorimeter and then diluted to 1 ng/μl. The size of the insert was checked using an Agilent bioanalyzer and further quantified by qPCR (library concentration > 2 nM). An aliquot (0.5 nM) of the pool was loaded on a high-output flow cell and sequenced on a NovaSeq 6000 instrument (Illumina) with 2 × 150 bp paired-end chemistry in two runs. Reads were aligned to human genome release hg38 using HISAT2 V2.0.5 with default parameters. Quantification of the expressed genes was performed using CUFFDIFF v2.2.1. The quality of the RNA-Seq count data was assessed using the Novogene standard protocol. The RNA-Seq data presented in this article was submitted to the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) under the accession number (GSE166617).

**Data mining**

TCGA/SKCM RNAseq data were analyzed using the OncoLnc portal [http://www.oncolnc.org] (Anaya, 2016). The raw data count matrix, composed of 454 samples (from SKCM melanoma cohort), was downloaded from the OncoLnc portal for the various transcriptional signatures. Expression heatmaps of differentially expressed genes between samples were obtained based on a log2 fold change using the ComplexHeatmap 2.0.0 (Gu et al., 2016) package in R/Bioconductor. Cluster-specific gene rankings were obtained by contrasting the samples with the rest. Cell density curves for the available melanoma cell lines were established using GraphPad PRISM 9.0® to establish the IC50 for the various treatments.

The raw data count matrices from the RNA seq data were obtained in GEO database for previous experiments on melanoma
cell lines (Barretina et al., 2012) GSE36134 [https://www.ncbi.nlm.nih.gov/gds/?term=GSE36134] (sensitive or resistant to PLX470; IC50 values for PLX4720 were obtained from Supplementary Table S7 of Barretina et al., 2012). BRAFi− or BRAFi+/MEKi-resistant cell lines GSE75299 [https://www.ncbi.nlm.nih.gov/gds/?term=GSE75299 (Song et al., 2017)] and GSE80829 [https://www.ncbi.nlm.nih.gov/gds/?term=GSE80829 (Tsoi et al., 2018)] and GSE110054 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE110054 (Tsoi et al., 2018)], BRAFi-treated melanoma patients GSE65185 [https://www.ncbi.nlm.nih.gov/gds/?term=GSE65185 (Hugo et al., 2015)] and melanoma cell lines (proliferative or invasive) GSE60664 [https://www.ncbi.nlm.nih.gov/gds/?term=GSE60664 (Verfallie et al., 2015)]. Analysis of the RNAseq dataset from the GDSC (Sanger/Massachusetts General Hospital Genomics of Drug Sensitivity in Cancer; Yang et al., 2013) was performed and recovered from the CellMinerCDB webtool [https://discover.nci.nih.gov/cellminercdb; Reinhold et al., 2012]. CellMinerCDB is an interactive web application that simplifies access to and exploration of cancer cell line pharmacogenomic data from different sources. This webtool allows the comparison of molecular and/or drug response patterns across sets of cell lines to search for possible associations. Pearson’s correlations with the reported p-values (not adjusted for multiple comparisons) between AhR expression (Appendix Fig S7) and drug activity (297 compounds) were recovered for various cancer cell lines (n = 1,080).

Statistics

Data are presented as the mean ± SD, unless otherwise specified, and differences were considered significant for a P value < 0.05. Comparisons between groups normalized to a control were carried out using a two-tailed t-test, with the Holm–Sidak multiple comparisons test when more than two groups are compared with the same control condition. Overall survival was estimated using the Kaplan–Meier method. Univariate analysis using the Cox regression model was performed to estimate the hazard ratios (HRs) and 95% confidence intervals (CI). All statistical analyses were performed using GraphPad (PRISM9.0®; La Jolla, CA, USA).

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. RNA-Seq data: Gene Expression Omnibus GSE166617 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166617].

Expanded View for this article is available online.

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Author contributions

Anaïs Paris: Formal analysis; investigation; methodology. Nina Tardif: Investigation; methodology. Francesca M Baietti: Investigation. Cyrille Berra: Investigation. Héloïse M Leclerc: Investigation. Eleonora Leucci: Investigation. Marie-Dominique Galibert: Supervision; funding acquisition; writing – original draft; project administration; writing – review and editing.

Sébastien Corre: Conceptualization; resources; formal analysis; supervision; funding acquisition; validation; investigation; writing – original draft; project administration; writing – review and editing.

Disclosure and competing interests statement

The authors declare that they have no conflict of interest.

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