MOLECULAR DETERMINANTS OF RESISTANCE TO TREATMENT WITH BRAF INHIBITORS AND INNOVATIVE POSSIBILITIES OF ITS OVERCOMING

MOLEKULARNE PODSTAWY OPORNOŚCI NA LECZENIE INHIBITORAMI BRAF ORAZ INNOWACYJNE MOŻLIWOŚCI JEJ POKONYWANIA

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Summary: The BRAF mutation is one of the alteration responsible for carcinogenesis of various tissues. It occurs notably more often in melanoma, but also is detected in papillary thyroid cancer, hairy cell leukemia and Langerhans cell histiocytosis. The occurrence of mutation leads to an increased BRAF kinase activity of the MAPK pathway in tumor cells. Targeted therapies based on the application of BRAF kinase inhibitors seem to be an effective treatment option of oncological conditions. However, a significant problem of monotherapy with these compounds are the frequent recurrences in patients within the first year after treatment initiation.

The described phenomenon is caused by the resistance to the treatment with MAPK pathway inhibitors. The resistance to BRAF inhibitors may be distinguished to primary and secondary – acquired during treatment. The problem appears additionally complicated due to the various nature and heterogeneous causes of the resistance. Moreover, the knowledge about the resistance is still growing, due to the continuous influx of new scientific reports. However, there is a noticeable tendency that the activation of alternative pathways is the leading cause of resistance.

The resistance to MAPK pathway inhibitors can be also divided into genetically or phenotypically determined. The most important genetic factors leading to resistance are mutations related to the modification of MAPK pathway proteins as well as PI3K/PTEN. Epigenetic modifications and other genomic modifications, are also significant. Phenotypic-based resistance may be associated with cells presenting elevated CRAF activation, alternative BRAF splicing or MITF gene amplifications.

This review includes comprehensive information on current reports, both on experimental and clinical capabilities to overcome drug resistance to inhibitors of the MAPK pathway. The molecular basis of the above-described phenomenon is still not clearly understood, and there is a strong need to solve this problem. Here, we compile the current fast-developing knowledge on this significant issue.

Keywords: BRAF inhibitors, drug resistance, MAPK, MITF

Streszczenie: Obecność mutacji BRAF jest jednym ze zjawisk odpowiedzialnych za kancerogenezę. Szczególnie często występuje w czerniaku, ale również wykrywa się ją w raku brodawkowatym tarczycy, białaczce włochatokomórkowej oraz histiocytozie z komórek Langerhansa. Występowanie mutacji prowadzi do podwyższonej aktywności kinazy BRAF w szlaku MAPK w komórkach nowotworowych. Terapie celowane polegają na aplikacji pacjentowi inhibitorów kinazy BRAF i wydają się być skuteczne. Jednakże istotnym problemem monoterapii tymi związkami jest częste występowanie wznów u pacjentów już po pierwszym roku od rozpoczęcia leczenia. Zjawisko to wynika z występowania oporności na leczenie inhibitorami szlaku MAPK.
Opisane powyżej zjawisko jest niezwykle istotne dla rokowania pacjentów z guzami wykazującymi mutacje BRAF. Oporność na inhibitory BRAF dzieli się na pierwotną oraz na wtórną tj. nabytą w trakcie leczenia tymi lekami. Problem jest dodatkowo skomplikowany ze względu na niejednorodny charakter oraz heterogenne przyczyny wystąpienia owego problemu. Ponadto, wiedza na temat oporności wciąż się rozwija z powodu pojawiających się stale nowych odkryć dotyczących mechanizmów molekularnych odpowiedzialnych za aktywność inhibitorów BRAF. Można zauważyć, że aktywacja alternatywnych szlaków jest dominującą przyczyną wystąpienia oporności. Oporność tą można podzielić ją pod kątem molekularnym na uwarunkowaną genetycznie lub fenotypowo.
Do ważniejszych czynników genetycznych prowadzących do oporności należą mutacje prowadzące do modyfikacji białek szlaków MAPK oraz PI3K/PTEN. Znaczące są także modyfikacje epigenetyczne oraz genomowe. Oporność uwarunkowana fenotypowo może być związana z obecnością komórek wykazujących podwyższoną aktywność CRAF, alternatywnego splicingu BRAF czy amplifikacji genu MITF.
W artykule opisano informacje uwzględniają aktualne doniesienia o doświadczalnych jak i klinicznych możliwościach przełamywania lekooporności w przypadku wystąpienia odporności na inhibitory szlaku MAPK. Molekularne podstawy opisanego zjawiska nie są wciąż całkowicie poznane, stąd konieczność rozwiązania tego problem.

Słowa kluczowe: inhibitory BRAF, oporność lekowa, MAPK, MITF

BRAF MUTATION

The presence of BRAF mutations in tumors is currently focusing scientists and clinicians around the world. The knowledge about this anomaly is dynamically developing, particularly in pharmacological sciences, where this genetical defect can be efficiently applied in the modern targeted therapies [23].

EPIDEMIOLOGY

The occurrence of BRAF mutations is different for individual cancers (Davies et al. 2002). Available data indicates that BRAF mutation is present in 6% of human cancers and 40-50% of melanoma cases[8]. However, these mutations have also been identified in papillary thyroid cancer (Fuziwara et al. 2019), hairy cell leukemia (Kreitman and Arons 2018), Langerhans cell histiocytosis [17], CRC and

NSCLC [8]. Subsequent studies have reported that this mutation frequent occurs in nevi and melanoma cases. The most often is BRAFV600E and BRAFV600K mutation, which is the most frequent genetic alteration of melanoma [6]. In Poland over half of melanomas (57%) display overexpression of that mutation [31].

The presence of BRAF-V600E mutation in thyroid cancer is also crucial, especially in the papillary type of this cancer. Xie et al. reported that in Papillary Thyroid Cancer (PTC) this mutation rates occurs in 62.1% (59/95) of cases [59]. BRAF mutations were also observed in lymphoproliferative malignancies. Moreover, in more than half of all LCH (Langerhans Cell Histiocytosis) BRAF-V600E mutation is detectable [25]. However, the highest percentage of BRAF mutations in tumors occurs in Hairy Cell Leukemia (HCL). The incidence of BRAF-V600E HCL is almost 100% [1], which explains the success of therapy with BRAF inhibitors in its treatment [51].

MOLECULAR DETERMINANTS OF OCCURRENCE

BRAF kinase is 766-amino acid, signal transduction serine/threonine-specific protein kinase which is part of the RAS-RAF-MEK-ERK pathway. *BRAF* gene in human is localized within the chromosome 7 [27]. In the cell, the RAS-RAF-MEK-ERK pathway is responsible for proliferation, cell motility and induction of apoptosis. Most of the mutations occurring in BRAF gene are observed in codon 600. These mutations are mainly a missense mutation, consisting of the replacement of a nucleotide, leading to the exchange of amino acids in the primary protein structure. The most frequent is BRAFV600E – where valine is substituted by glutamic acid [52]. The other common is BRAFV600K – the substitution by lysine [29]. The described abnormalities lead to the constitutive activity of BRAF kinase, and thus the continuous activity of the MAPK (mitogen-activated protein kinases), which is the cause of aggressive cell transformation and growth [19].

THE HISTORY OF BRAF INHIBITORS

Over the recent years MAPK inhibitors become an important target on the field of molecularly targeted drug discovery industry in experimental oncology. However, studies concerning RAS inhibitor discovery become unsuccessful [38], in contrast to studies concerning RAF kinase inhibitor. The first BRAF-targeted drug was sorafenib. This agent inhibits BRAF activity by binding to a specific site instead of ATP on this kinase. The effectiveness of the drug was proven, among others on HepG2 and Huh7 cell line *in vitro* [3] and advanced hepatocellular carcinoma in clinical application [5].

The main disadvantage of this drug is low selectivity level of this agent. Sorafenib is a multikinase inhibitor which is able to inhibit simultaneously all of the tyrosine kinases in the cell among others VEGFR, PDGFR, FLT-3 and p38 MAP [58, 16].

The discovery of vemurafenib became the breakthrough in selective cytostatics development. Flaherty et al. in 2010 revealed PLX4032 (precursor of vemurafenib) inducing complete or partial tumor regression in 81% of patients suffering from BRAFV600E-mutated melanoma [13]. Within the next few years, more selective BRAFV600E inhibitors were discovered – such as dabrafenib or encorafenib [32]. Despite the successful usage of this agent in different cancer management, a large number of patients has developed acquired resistance during the treatment and relapse. One of the solution to this problem can be combined therapy. It has been reported that combination of dabrafenib plus trametinib increases overall survival in patients suffering from BRAFV600E/BRAFV600K melanoma as compared to vemurafenib monotherapy [44]. However, the resistance is still occurring even in the implementation of the combined therapy [30].

THE FREQUENCY OF RESISTANCE TO BRAF INHIBITORS (BRAFI)

The resistance to MAPK inhibitors is a statistically significant phenomenon. Almost 50% of BRAF mutated melanomas are therapeutically attackable for MAPK inhibitor [10]. Furthermore, despite frequently initial response to the treatment with BRAFi after 4-15 months resistance appears [54]. This problem is particularly important for modern medicine because it affects drastically the average survival of patients [48].

MAPK REACTIVITY RELATED RESISTANCE

In case of MAPK related resistance the lack of BRAFi effectiveness is based on bypassing of inhibited BRAF by obtaining MEK activation in BRAF-independent manner, which leads to ERK-dependent signaling restoration. The figure 1 summarizes the main MAPK-related mechanisms, which are described below.

Alternative splicing variants of BRAFV600E were the first identified mechanisms of resistance to BRAF inhibitors. Their frequent occurrence is considered to be clinically important. Nevertheless, the available data suggest that the addition of MEK inhibitors to the treatment with BRAFi could delay or prevent above-mentioned type of resistance [43]. BRAF amplifications are a most common cause of resistance to BRAFi. Yaeger et al. have shown that 1.7 – fold amplification of RAS is associated with a noticeable reduction in the inhibition of ERK phosphorylation for

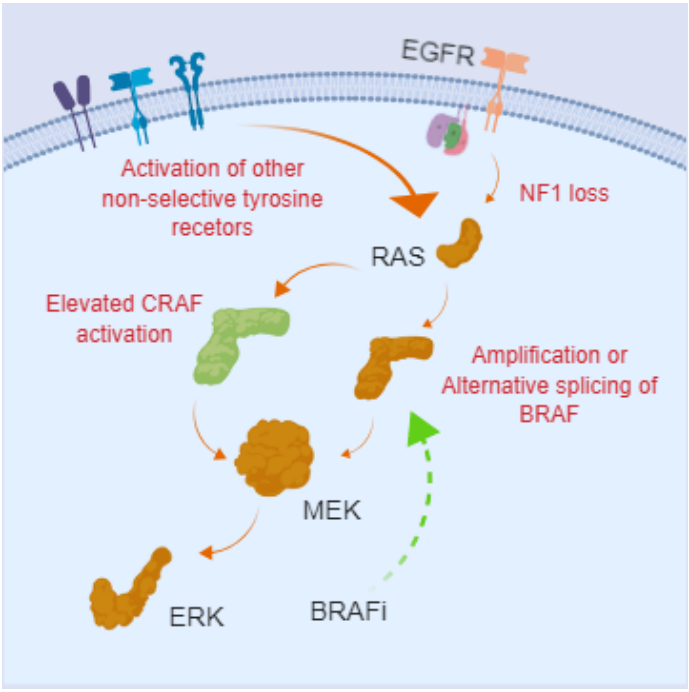

FIGURE 1. Molecular basics of MAPK-related BRAFi resistance (created with BioRender.com and based on the [55])
RYCINA 1. Molekularne uwarunkowania MAKP-zależnej oporności na inhibitory BRAF ([55])

one hour of vemurafenib/cetuximab treatment [60]. There was indicated that NF1 has been playing a very important role in cell metabolism and the product of the NF1 gene is neurofibromin, which major known function is to downregulate RAS [42]. There was also noticed that NF1 alteration may confer resistance to BRAF inhibition in human melanoma. Furthermore, it was revealed that these changes may be responsible for both *de novo* and acquired resistance to vemurafenib [57].

The next important mechanism occurs, because mitogen-activated protein kinases can be activated simultaneously with other different receptor-linked kinases. After the binding of specific ligand to this receptor, at the cytoplasmic side tyrosine kinase becomes activated and it leads to cascade reactions and MEK activation [33]. The described process may be responsible for the resistance to BRAFi [50]. The mentioned surface receptors are able to take part in the same processes as EGFR, Trk A/B, FGFR and PDGFR [53]. Based on this information, it seems understandable why dual EGFR and BRAF blockade overcomes resistance to BRAFi. That was revealed that the dominated therapy with PLX4032 (vemurafenib) and gefitinib resulted in synergistic action in the BRAF therapy of mutated thyroid carcinoma [36].

Also important is occurrence of resistance to vemurafenib by MEK activation due to overexpression or phosphorylating of CRAF kinase. This phenomenon is based on bypassing the inhibited BRAF kinase what leads to the activation of ERK [30]. An effective method to overcome this type of resistance may be the usage of HSP90 inhibitor like XL888 which was reported to overcome BRAF inhibitors resistance [40].

NON-MAPK REACTIVITY RELATED RESISTANCE

In case of the resistance to non-MAPK related BRAF inhibitors cell survival is obtained by using alternative pathways and by-passing inhibited MAPK pathway. The fig. 2 shows the main non-MAPK related mechanisms which are described below.

The activation of the PI3K/AKT pathway is one of the MAPK-independent mechanism responsible for relapse during treatment with BRAF/MEK inhibitors. However, it is worth to mention that PI3K/AKT activity promotes survival, but is not responsible for proliferation in response to dominated BRAF/MEK inhibitor

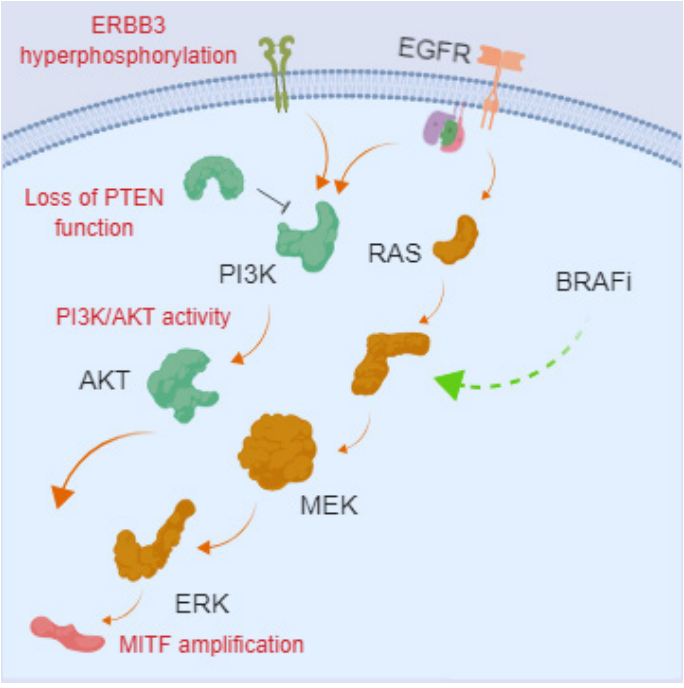

FIGURE 2. Molecular basics of non-MAPK-related BRAFi resistance (created with BioRender.com and based on the [22])
RYCINA 2. Molekularne uwarunkowania MAKP-niezależnej oporności na inhibitory BRAF ([22])

treatment [21]. It was also proved that resistance to PLX4720 (BRAFi) may be mediated through AKT/DAB pathway activation [41]. The constant activation of AKT3 could protect BRAF kinase from inhibition by BRAFi [45]. Furthermore, activation of the AXL/AKT axis may also be responsible for that kind of resistance [61]. Studies suggest that AKT3 could be activated in 40-60% of melanoma [4]. Taking it all into account – the data suggests that alteration in PI3K/AKT pathway are important targets in case of resistant to BRAFi. It was reported that combination HDAC (histone deacetylase) inhibitor – panobinostat – may overcome BRAFi resistance by reduction of PI3K pathway activity [15]. Besides, Greger et al. studies presented that the combination of GSK2126458 (PI3K/mTOR inhibitor) and MAPK inhibition enhanced cell growth inhibition in cell lines with acquired resistance [34]. Finally, it was shown that GSK2141795B (AKTi) was able to the emergence of resistance or enhance the activity of BRAFi [28]. In the other studies was also observed that loss of PTEN may be responsible for BRAFi resistance occurrence due to suppression of BIM expression. Paraiso et al. studies revealed that this alteration occurs in more than 10% of melanoma cases. Due to the fact that the main role of PTEN is to inhibit the phosphorylation of PI3K, an explainable phenomenon is that combination of BRAFi with PI3Ki enhances BIM expression and proapoptotic effect [39]. The following mechanism responsible for resistance is up-regulation of phosphor-ERBB3, which is the result of feedback autocrine loop involving increased transcription and production of neuregulin [11]. It has been proved that activation of this receptor by neuregulin may induce the escape from the cytotoxic effect of BRAFi [35]. However, this mechanism could be abrogated by the addition of monoclonal antibodies against ERBB3 [12].

Finally, MITF gene amplification is also responsible for BRAFi resistance. Microphthalmia-associated transcription factor (MITF) is associated with pigmentation, DNA replication and proliferation. MITF is the key transcriptional factor responsible for melanocyte differentiation, development, homeostasis and cell cycle promotion, which explains the role of MITF gene alteration in the oncogenesis of some cases of melanoma [24]. MITF gene alterations are responsible for MAPKi resistance and this phenomenon is PAX3-reated. Smith et al. studies show that nelfinavir affects PAX3 and MITF, through among others inducing SMAD2/4/SKI repressor complex, and sensitizes to MAPK inhibition in BRAF mutant melanoma and where c.a. 80% volume reduction was achieved [46].

STROMAL CELL INFLUENCE

In the case of the BRAF inhibitor resistance dependent on the stromal cell, the cell survival is obtained via different mechanism induced by specific mediators. These mediators are secreted by cell from tumor microenvironment in response to

BRAFi influence as it is shown in fig.3 [37]. The role of the stromal cells in this process is rather not clarified and undetected. Though, the secreted matricellular protein connective tissue growth factor (CCN2) was pointed as a potential therapeutic candidate to block an activation of connective tissue in fibrosis and cancers and therapeutic target for BRAF inhibitor-resistant melanoma [20].

Thus fibroblasts play the important role, and are able to remodel the environment of BRAF mutated tumor and, in this way, promote the resistance to MAPK inhibitors. Melanoma cells in response to the BRAF inhibitors secrete TGF-β (transforming growth factor β). TGF-β stimulates fibroblasts to the secretion of fibronectin with integrin β1 remodeling and phosphorylation of focal adhesion kinase (FAK). Therefore, activated FAK induces ERK phosphorylation which contributes to BRAF inhibitor resistance [34]. Another proposed mechanism of resistance is related to HGF (hepatocyte growth factor) production by the tumor stroma [18]. Straussman et al. studies indicated that HGF-induced resistance is greater when is induced by BRAFi than by MEKi. Furthermore, the combination of BRAFi and MEKi is not sufficient to eliminate resistance because here AKT is not inhibited. The only combination of MEKi and AKT inhibitors is able to inhibit the majority of HGF-induced resistance [49].

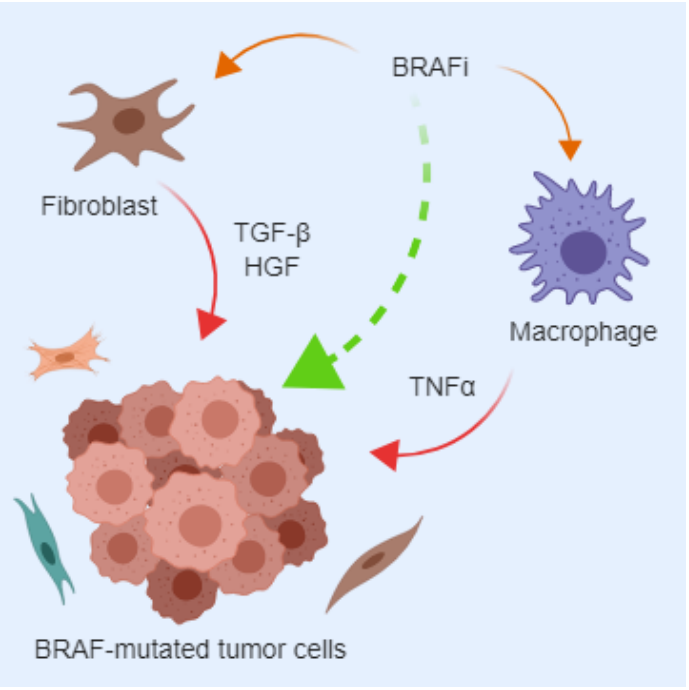

FIGURE 3. Molecular basics of stromal cell-related BRAFi resistance (created with BioRender.com and based on [2])
RYCINA 3. Molekularne uwarunkowania oporności na inhibitory BRAF zależnej od wpływu komórek podścieliska guza ([2])

The next stromal cells involved in this phenomenon are macrophages. BRAFi resistance related to its activity is mediated by different factors. The key factor involved in resistance related to TNFα (tumor necrosis factor alfa). It has been reported that this compound is required to grow and survival of melanoma cells. TNFα up-regulates MITF in melanoma cells, what suppressed MEKi induced caspase3 cleavage. However, the same study revealed that IKK inhibition may suppress TNFα secretion and overcome this mechanism of resistance [47]. The second important factor is VEGF (vascular endothelial growth factor). The phenomenon of this type of resistance is based on the paradoxical activation of MAPK in macrophages treated with BRAF inhibitors, what promote VEGF secretion, what then leads to MAPK reactivation in melanoma cells and escape from BRAFi response [56]. Comunanza et al. studies suggest that targeting VEGF may enhance the antitumor effect of BRAFi by inducing vascular normalization, by-passing immune tolerance and influencing on CAFs [7].

CONCLUSIONS

The basis of the BRAF-mutated cells resistance of to BRAF inhibitors is multifactorial and is still not fully investigated. The available reports focus how to overcome this phenomena using combined therapies (e.g. with other inhibitors of MAPK or PI3K). Thus, the development of next-generation therapies, searching for the new mechanisms and protocols which may be implemented to break the resistance to BRAF inhibitors, are the challenging tasks for the future studies.

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REFERENCES

[1] Ahmadzadeh A, et al. BRAF Mutation in Hairy Cell Leukemia. *Oncol Rev.* 2014; **8**(2): 253.
[2] Arozarena I, Wellbrock C. Overcoming Resistance to BRAF Inhibitors. *Ann Transl Med.* 2017; **5**(19): 387.
[3] Cervello M, et al. "Molecular Mechanisms of Sorafenib Action in Liver Cancer Cells." *Cell Cycle.* 2012; **11**(15):2843-55.
[4] Chan, Yang X, Singh A, Osman N, Piva T. "Role Played by Signalling Pathways in Overcoming BRAF Inhibitor Resistance in Melanoma." *International Journal of Molecular Sciences.* 2017; **18**(7).

[5] Cheng A,et al. “Efficacy and Safety of Sorafenib in Patients in the Asia-Pacific Region with Advanced Hepatocellular Carcinoma: A Phase III Randomised, Double-Blind, Placebo-Controlled Trial.” *The Lancet Oncology.* 2009; **10**(1): 25-34.

[6] Cheng L, Lopez-Beltran A, Massari F, Maclennan G, Montironi R“Mol. ecular Testing for BRAF Mutations to Inform Melanoma Treatment Decisions: A Move toward Precision Medicine.” Modern Pathology : *An Official Journal of the United States and Canadian Academy of Pathology, Inc.* 2018; **31**(1): 24-38.

[7] Comunanza V, et al. “VEGF Blockade Enhances the Antitumor Effect of BRAFV600E Inhibition.” EMBO *Molecular Medicine.* 2017; **9**(2): 219-37.

[8] Dankner M, Rose A, Rajkumar S, Siegel P, Watson I “Classifying BRAF Alterations in Cancer: New Rational Therapeutic Strategies for Actionable Mutations.” *Oncogene.* 2018; **37**(24): 3183-99.

[9] Davies H, et al. “Mutations of the BRAF Gene in Human Cancer.” *Nature*. 2002; **417**(6892): 949-54.

[10] Dietrich, Kuphal P, Spruss T, Hellerbrand C, Bosserhoff A “Wild-Type KRAS Is a Novel Therapeutic Target for Melanoma Contributing to Primary and Acquired Resistance to BRAF Inhibition.” *Oncogene.* 2018; **37**(7):897-911.

[11] Fattore L, et al. “Activation of an Early Feedback Survival Loop Involving Phospho-ErbB3 Is a General Response of Melanoma Cells to RAF/MEK Inhibition and Is Abrogated by Anti-ErbB3 Antibodies.” *Journal of Translational Medicine.* 2013; **11**(1): 180.

[12] Fattore L, et al. “Combination of Antibodies Directed against Different ErbB3 Surface Epitopes Prevents the Establishment of Resistance to BRAF/MEK Inhibitors in Melanoma.” *Oncotarget.* 2015; **6**(28): 24823-41.

[13] Flaherty KT, et al. “Inhibition of Mutated, Activated BRAF in Metastatic Melanoma.” *New England Journal of Medicine.* 2010; **363**(9): 809-19.

[14] Fuziwara SC, Saito K, Leoni S, Waitzberg A, Kimura E “The Highly Expressed FAM83F Protein in Papillary Thyroid Cancer Exerts a Pro-Oncogenic Role in Thyroid Follicular Cells.” *Frontiers in Endocrinology.* 2019; **10**: 134.

[15] Gallagher SJ, et al. “HDAC Inhibitors Restore BRAF-Inhibitor Sensitivity by Altering PI3K and Survival Signalling in a Subset of Melanoma.” *International Journal of Cancer.* 2018; **142**(9): 1926-37.

[16] Haas NB, et al. “Weekly Bryostatin-1 in Metastatic Renal Cell Carcinoma: A Phase II Study.” Clinical Cancer Research : *An Official Journal of the American Association for Cancer Research* 2003, **9**(1):109-14.

[17] Héritier S, et al. “BRAF Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy.” *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology.* 2016; **34**(25): 3023-30.

[18] Hirata E, et al. “Intravital Imaging Reveals How BRAF Inhibition Generates Drug-Tolerant Microenvironments with High Integrin β1/FAK Signaling.” *Cancer Cell.* 2015; **27**(4): 574-88.

[19] Hugdahl E, May Britt Kalvenes, Hanne E. Puntervoll, Rita G. Ladstein, And Lars A. Akslen. “BRAF-V600E Expression in Primary Nodular Melanoma Is Associated with Aggressive Tumour Features and Reduced Survival.” *British Journal of Cancer.* 2016; **114**(7): 801-8.

[20] Hutchenreuther J, Leask A. “Why Target the Tumor Stroma in Melanoma?” *Journal of Cell Communication and Signaling.* 2018; **12**(1): 113-18.

[21] Irvine, M, et al. “Oncogenic PI3K/AKT Promotes the Step-Wise Evolution of Combination BRAF/MEK Inhibitor Resistance in Melanoma.” *Oncogenesis.* 2018; **7**(9): 72.

[22] Kakadia S, et al. “Mechanisms of Resistance to BRAF and MEK Inhibitors and Clinical Update of US Food and Drug Administration-Approved Targeted Therapy in Advanced Melanoma.” *OncoTargets and Therapy.* 2018; **11**: 7095-7107.

[23] Karoulia Z, Gavathiotis E, Poulikakos P. “New Perspectives for Targeting RAF Kinase in Human Cancer.” *Nature Reviews. Cancer* 2017; **17**(11): 676-91.

[24] Kawakami A, Fisher D. “The Master Role of Microphthalmia-Associated Transcription Factor in Melanocyte and Melanoma Biology.” *Laboratory Investigation.* 2017; **97**(6): 649-56.

[25] Kobayashi M, Tojo A. "Langerhans Cell Histiocytosis in Adults: Advances in Pathophysiology and Treatment." *Cancer Science.* 2018; **109**(12): 3707-13.
[26] Kreitman RJ, Evgeny A. "Update on Hairy Cell Leukemia." *Clinical Advances in Hematology & Oncology : H&O.* 2018; **16**(3):.205-15.
[27] Lassaletta A, et al., "Therapeutic and Prognostic Implications of BRAF V600E in Pediatric Low-Grade Gliomas." *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology.* 2017; **35**(25): 2934-41.
[28] Lassen A, et al. "Effects of AKT Inhibitor Therapy in Response and Resistance to BRAF Inhibition in Melanoma." *Molecular Cancer.* 2014; **13**(1): 83.
[29] Li Y, David M. Umbach, Leping Li. "Putative Genomic Characteristics of BRAF V600K versus V600E Cutaneous Melanoma." *Melanoma Research.* 2017; **27**(6):527-35.
[30] Lu H, et al. "PAK Signalling Drives Acquired Drug Resistance to MAPK Inhibitors in BRAF-Mutant Melanomas." *Nature.* 2017; **550**(7674): 133-36.
[31] Mackiewicz-Wysocka, M, et al. "Oncogenic BRAF Mutations and p16 Expression in Melanocytic Nevi and Melanoma in the Polish Population." *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii.* 2017; **34**(5): 490.
[32] Mackiewicz J, Mackiewicz A "BRAF and MEK Inhibitors in the Era of Immunotherapy in Melanoma Patients." *Contemporary Oncology.* 2018; **22**(1A): 68-72.
[33] Mccubrey JA, et al. "Roles of the Raf/MEK/ERK Pathway in Cell Growth, Malignant Transformation and Drug Resistance." *Biochimica et Biophysica Acta.* 2007; **1773**(8): 1263-84.
[34] Menon RD, Schaider H. "Microenvironment-Driven Resistance to BRAF Inhibition Comes of Age*."The Journal of Investigative Dermatology.* 2015; **135**(12): 2923-25.
[35] Ng Y-K, et al. "Pan-erbB Inhibition Potentiates BRAF Inhibitors for Melanoma Treatment." *Melanoma Research.* 2014; **24**(3): 207-18.
[36] Notarangelo T , Sisinni L, Condelli V, Landriscin M. "Dual EGFR and BRAF Blockade Overcomes Resistance to Vemurafenib in BRAF Mutated Thyroid Carcinoma Cells." *Cancer Cell International.* 2017; **17**(1): 86.
[37] Owusu YB, Galemmo R, Janetka J, Klampfer L. "Hepatocyte Growth Factor, a Key Tumor-Promoting Factor in the Tumor Microenvironment." *Cancers.* 2017; **9**(4).
[38] Papke B, Der ChJ. "Drugging RAS: Know the Enemy." *Science.* 2017; (New York, N.Y.) **355**(6330) :1158-63.
[39] Paraiso KHT, et al. "PTEN Loss Confers BRAF Inhibitor Resistance to Melanoma Cells through the Suppression of BIM Expression." *Cancer Research.* 2011; **71**(7): 2750-60.
[40] Paraiso KHT, et al. "The HSP90 Inhibitor XL888 Overcomes BRAF Inhibitor Resistance Mediated through Diverse Mechanisms." *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research.* 2012; **18**(9): 2502-14.
[41] Perna D, et al. "BRAF Inhibitor Resistance Mediated by the AKT Pathway in an Oncogenic BRAF Mouse Melanoma Model." *Proceedings of the National Academy of Sciences of the United States of America.* 2015; **112**(6): E536-45.
[42] Philpott Ch, Tovell H, Frayling I, Cooper D, Upadhyaya M. "The NF1 Somatic Mutational Landscape in Sporadic Human Cancers." *Human Genomics.* 2017; **11**(1): 13.
[43] Poulikakos PI, et al. "RAF Inhibitor Resistance Is Mediated by Dimerization of Aberrantly Spliced BRAF(V600E)." *Nature.* 2011; **480**(7377): 387-90.
[44] Robert C, et al. "Improved Overall Survival in Melanoma with Combined Dabrafenib and Trametinib." *New England Journal of Medicine.* 2015; **372**(1): 30-39.
[45] Shao Y, Aplin A "Akt3-Mediated Resistance to Apoptosis in B-RAF-Targeted Melanoma Cells." *Cancer Research* 2010; **70**(16): 6670-81.
[46] Smith MP, et al. "Inhibiting Drivers of Non-Mutational Drug Tolerance Is a Salvage Strategy for Targeted Melanoma Therapy." *Cancer Cell.* 2016; **29**(3): 270-84.
[47] Smith MP, et al. "The Immune Microenvironment Confers Resistance to MAPK Pathway Inhibitors through Macrophage-Derived TNFα." *Cancer Discovery.* 2014; **4**(10): 1214-29.

[48] Spagnolo F, et al. "BRAF-Mutant Melanoma: Treatment Approaches, Resistance Mechanisms, and Diagnostic Strategies." *OncoTargets and Therapy.* 2015; **8**: 157-68.
[49] Straussman R, et al. "Tumour Micro-Environment Elicits Innate Resistance to RAF Inhibitors through HGF Secretion." *Nature.* 2012; **487**(7408): 500-504.
[50] Sun Ch, et al. "Reversible and Adaptive Resistance to BRAF(V600E) Inhibition in Melanoma." *Nature* 2014; **508**(7494): b118-22.
[51] Tiacci E, et al. "Targeting Mutant BRAF in Relapsed or Refractory Hairy-Cell Leukemia." *The New England Journal of Medicine* 2015; **373**(18): 1733-47.
[52] Tsao H, Chin L, Garraway L, Fisher D. "Melanoma: From Mutations to Medicine." *Genes & Development* 2012; **26**(11): 1131-55.
[53] Vella LJ, et al. "Intercellular Resistance to BRAF Inhibition Can Be Mediated by Extracellular Vesicle-Associated PDGFRβ." *Neoplasia* 2017; **19**(11): 932-40.
[54] Villanueva J, et al. "Acquired Resistance to BRAF Inhibitors Mediated by a RAF Kinase Switch in Melanoma Can Be Overcome by Cotargeting MEK and IGF-1R/PI3K." *Cancer Cell*. 2010; **18**(6): 683-95.
[55] Villanueva J, Vultur A, Herlyn M. "Resistance to BRAF Inhibitors: Unraveling Mechanisms and Future Treatment Options." *Cancer Research.* 2011; **71**(23): 7137-40.
[56] Wang T, et al. "BRAF Inhibition Stimulates Melanoma-Associated Macrophages to Drive Tumor Growth." *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research.* 2015; **21**(7): 1652-64.
[57] Whittaker SR, et al. "A Genome-Scale RNA Interference Screen Implicates NF1 Loss in Resistance to RAF Inhibition." *Cancer Discovery.* 2013; **3**(3): 350-62.
[58] Wilhelm SM. et al. "BAY 43-9006 Exhibits Broad Spectrum Oral Antitumor Activity and Targets the RAF/MEK/ERK Pathway and Receptor Tyrosine Kinases Involved in Tumor Progression and Angiogenesis." *Cancer Research.* 2004; **64**(19): 7099-7109.
[59] Xie H, et al. "BRAF Mutation in Papillary Thyroid Carcinoma (PTC) and Its Association with Clinicopathologi; cal Features and Systemic Inflammation Response Index (SIRI)." *American Journal of Translational Research* 2018; **10**(8): 2726-36.
[60] Yaeger R, Yao Z, Hyman DM, et al. "Mechanisms of Acquired Resistance to BRAF V600E Inhibition in Colon Cancers Converge on RAF Dimerization and Are Sensitive to Its Inhibition. *Cancer Res.* 2017; **77**(23): 6513-6523.
[61] Zuo Q, et al. "AXL/AKT Axis Mediated-Resistance to BRAF Inhibitor Depends on PTEN Status in Melanoma." *Oncogene.* 2017; **37**(24): 3275-89.

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