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Anticipating designer drug-resistant cancer cells

Q1 Mark L. Frangione[1], John H. Lockhart[1], Daniel T. Morton[1], Libia M. Pava[3] and George Blanck[1,2], gblanck@health.usf.edu

Successful use of anticancer designer drugs is likely to depend on simultaneous combinations of these drugs to minimize the development of resistant cancer cells. Considering the knowledge base of cancer signaling pathways, mechanisms of designer drug resistance should be anticipated, and early clinical trials could be designed to include arms that combine new drugs specifically with currently US Food and Drug Administration (FDA)-approved drugs expected to blunt alternative signaling pathways. In this review, we indicate examples of alternative signal pathways for recent anticancer drugs, and the use of original, Python-based software to identify systematically signaling pathways that could facilitate resistance to drugs targeting a particular protein. Pathway alternatives can be assessed at http://www.alternativesignalingpathways.com, developed with this review article.

Introduction

Q2 The past several years have seen the advent of patient treatments with drugs based on a molecular understanding of changes in cancer cells, including changes attributable to specific mutations and specific fusion genes. In some cases, resistance is a relatively minor issue and these drugs have proven to have long-term beneficial effects, as in the case of imatinib directed against the BCR-ABL fusion protein. In other cases, resistance occurs relatively rapidly. Given the general lack of specificity of intracellular signaling pathways that lead to specific cancer hallmarks, this result is not surprising. For example, classical tumor suppressor proteins and metastasis suppressor proteins differ very little, if at all, in their interactions with various signaling pathways linked to tumorigenesis [1]. In the case of vemurafenib, used to treat V600E BRAF melanomas, where the pro-cancer RAS signaling pathway is activated, resistance occurs rapidly via the activation of alternative signaling pathways [2].

Vemurafenib resistance in melanoma

V600E BRAF mutations are observed in approximately 60% of melanomas [3], making it a common therapeutic target for the small molecule inhibitor, vemurafenib. As noted above, rapidly acquired resistance mechanisms have been well documented in vemurafenib-treated melanomas [2]. As is the case for other drugs, such as imatinib [4], the overexpression of the ATP binding cassette drug transporter protein, ABCG2, has been observed as a mechanism of resistance to vemurafenib [5]. The anomalous activation of the signal transducer and activator of transcription 3 (STAT3) pathway is another mechanism of acquired resistance observed in V600E BRAF melanomas, representing the overactivation of a substitute, procancer signaling pathway. In this case, it is thought that vemurafenib stimulates the expression and secretion of fibroblast growth factor 2 (FGF2) from keratinocytes and fibroblasts in the tumor microenvironment [6].

However, other evidence suggests that this overactivation of STAT3 is mediated by upregulation of Ras-related C3 botulinum toxin substrate 1 (RAC1), in turn as a result of BRAF inhibition. Interestingly, melanoma invasiveness can be traced to vemurafenib inhibition of V600E BRAF signaling that leads to RAC1 upregulation, which facilitates metastasis by causing a switch in the expression of N-cadherin to E-cadherin [3]. Constitutively active RAC1, observed in vemurafenib-treated melanomas, as noted above,

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TABLE 1

Possible combination therapies based on alternative signaling pathways

Tumor type	Target protein and primary drug	Resistance mechanisms	Resistance mechanism comments	Possible drug combination using FDA-approved drugs[a]	Possible combinations at preclinical or clinical trial stage[a]	Refs
Melanoma	BRAF V600E; vemurafenib	Hypothesized by others: STAT3 activation	Possible linkage of BRAF/MEK/ERK and STAT3 pathways through RAC1, as proposed in this review	Anti-STAT3: WP1066	Anti-RAC1: 23A8	WP1066 [27,28]; 23A8 [29]
Glioblastoma	VEGF; bevacizumab	S100A4-secreting neutrophils and macrophage alter ECM and thereby provide alternate pathway to metastasis	Tumor hypoxia caused by VEGF inhibition induces myeloid infiltration		Anti-S100A4: mAb 5C3	mAb 5C3 and 24023743 [11]
B cell lymphoma	CD74; milatuzumab	Proposed in this review: ERK activation through CD44, and Akt activation through CD84	CD44 is signaling component with CD74 activated by MIF and phosphorylates ERK; CD84 is activated by CD74 and initiates survival pathway through AKT phosphorylation	Anti-CD44: A3D8	Anti-CD84: CD84.1.21	A3D8 [14]; anti-CD84 [30]
Breast	HER2; trastuzumab/ pertuzumab	Autophagy mechanisms; activation of ER signaling pathway	ER activation leads to growth and activation of PI3K/AKT pathway	Chloroquine		Chloroquine [23]
Basal cell carcinoma	SMO; vismodegib	Downstream activation of GLI-1 through mTOR/S6K1; PI3K activation through PTEN loss but no known connection to GLI-1	Through mTOR, S6K1 can prevent SUFU inhibition of GLI-1, thus activating transcription of targets downstream of GLI-1; PI3K upregulation because of loss of PTEN either through mutation or epigenetic gene inactivation		S6K1 inhibitor: PF4708671	PF4708671 [31]

[a] Refers only to possible future consideration for combination use.

leads to activation of the STAT3 pathway [7]; however, this latter mechanism has thus far only been observed in normal endothelial cells and not in cancer cells. Nevertheless, this raises the possibility that the activation of the STAT3 pathway observed in vemurafenib-resistant melanomas is mediated by overactivation of RAC1. Thus, we hypothesize that dual targeting the V600E mutant BRAF along with RAC1 could be a novel therapeutic strategy for treating melanomas with the V600E BRAF mutation (Table 1).

Bevacizumab resistance in glioblastoma

Vascular endothelial growth factor (VEGF) is a crucial mediator of angiogenesis, which enhances the invasiveness of cancers, particularly glioblastoma. The antiangiogenic monoclonal antibody (mAb) bevacizumab targets VEGF and has been partially successful in clinical trials [8]. Several mechanisms of resistance have been documented in glioblastomas treated with bevacizumab. Given the hypoxic environment, in turn resulting from decreased blood flow, tumors tend to shift towards anaerobic respiration and increased glycolysis, evidenced by an increase in lactate [8]. This has been observed in conjunction with a shift towards the pro-cancer phosphoinositide 3-kinase (PI3K)/AKT pathway as well as the WNT pathway [8]. Also, tumors treated with bevacizumab upregulate angiopoietin-2, prostaglandin-endoperoxide synthase 1, endothelial tyrosine kinase, urokinase, and VEGF-A, which suggests activation of alternative angiogenic pathways [8].

Bevacizumab and the 5C3 mAb

In conjunction with the hypoxic environment caused by anti-VEGF therapy, it has been observed that tumors become infiltrated with myeloid cells [9], including, S100A4-secreting neutrophils and macrophages [10]. S100A4 has also been shown to be involved with advancing tumor aggressiveness and invasiveness in other cancers by promoting angiogenesis synergistically with VEGF, altering cell adhesiveness, and upregulating the expression of matrix metalloproteinases (MMPs), thereby remodeling the extracellular matrix (ECM) [11]. Although this mechanism is not fully understood, the angiogenic association between S100A4 and VEGF makes S100A4 a novel therapeutic target. 5C3 is a mAb that binds S100A4 and has demonstrated positive results in reducing tumor growth in mice [11]. The 5C3 mAb is a strong candidate for human cancer treatments, and simultaneous targeting of metastasis with mAb 5C3 and bevacizumab could be a novel

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