Hedgehog Fights Back: Mechanisms of Acquired Resistance against Smoothened Antagonists

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Abstract

Acquired resistance to targeted therapies threatens the value of these otherwise very promising agents. The recent description of resistance to the Hedgehog pathway inhibitor vismodegib (GDC-0449) in a medulloblastoma patient who had a dramatic initial response has spurred efforts to understand potential mechanisms of drug resistance. Elucidating these mechanisms will play a significant role in informing strategies to overcome this meaningful limitation. Cancer Res; 71(15); 1–5. ©2011 AACR.

Introduction

The development of targeted molecular therapies, together with a growing emphasis on identifying patient populations most likely to respond to these therapies, is dramatically changing the landscape of medical oncology. However, as the field of targeted therapies matures, we are being forced to acknowledge an unfortunate but recurring theme: acquired drug resistance. It is becoming clear that understanding and even anticipating mechanisms of resistance to targeted therapies, and developing strategies to overcome them, will be critical for achieving long-term efficacy of targeted anticancer molecules in patients.

The theme of acquired drug resistance is particularly prevalent in the context of kinase inhibitors. Multiple mechanisms of resistance have been documented for a wide array of kinase antagonists, including imatinib, a BCR-ABL inhibitor that is perhaps the most intensively studied resistance model; erlotinib, an epidermal growth factor receptor (EGFR) inhibitor; and more recently crizotinib, an EML4-ALK inhibitor (1, 2). The recent introduction of the Hedgehog (Hh) pathway inhibitor vismodegib (also known as GDC-0449) to the clinic led to the first report of resistance against a therapy targeting Smoothened (SMO), and thus extended the paradigm of acquired drug resistance to G-protein coupled receptor (GPCR)-like molecules (3). This review focuses on mechanisms of resistance against Hh pathway inhibitors targeting SMO.

Hh Signaling Is an Enticing Therapeutic Target

Hh signaling is critical for embryonic development and also appears to play limited roles in adult homeostasis. In the resting state, the 12-pass transmembrane receptor Patched (PTCH) suppresses the activity of SMO, a GPCR-like molecule. The Hh ligand activates the pathway by binding to PTCH and releasing its inhibition over SMO, which subsequently signals to the GLI family of transcription factors and induces a change in the transcriptional profile of the cell (ref. 4; Fig. 1). Inappropriate activation of the Hh pathway is associated with cancer, a relationship that is best demonstrated in basal cell nevus (Gorlin) syndrome. Patients with Gorlin syndrome harbor germline mutations in PTCH1 that drive hyperactivation of the Hh pathway and are responsible for the development of numerous basal cell carcinomas throughout the lifetime of the patient (5). Patients with Gorlin syndrome are also predisposed to other types of cancer, in particular medulloblastoma, a tumor of cerebellar granule neuron progenitor cells. Analyses of sporadically occurring basal cell carcinomas and medulloblastomas have further highlighted a role for Hh signaling in these tumor types: Deregulation of PTCH1 contributes to hyperactive Hh signaling in the majority of sporadically occurring basal cell carcinomas, with activating mutations in SMO occurring in ~10% of cases (6). In medulloblastoma, the most common brain malignancy in young children, approximately one third of cases exhibit hyperactive Hh signaling due to mutations in components of the Hh pathway (7). In addition, mouse models have been used to reveal a causative role of the Hh pathway in medulloblastoma and basal cell carcinoma; for example, mice heterozygous for Pch (Pch+/−) were shown to spontaneously develop medulloblastoma (8).

The fact that hyperactive Hh signaling is clearly a driving force in medulloblastoma and basal cell carcinoma makes it a particularly enticing therapeutic target, much like hyperactive EGFR signaling made it a target of choice in non–small cell lung cancer (9). Adding to the considerable appeal of the Hh pathway as a therapeutic target is the discovery that cyclopamine, a natural compound derived from corn lilies that causes teratogenic effects in lambs, inhibits the Hh pathway by binding to SMO, providing evidence that the Hh pathway is amenable to inhibition by exogenous compounds (10). More recently, high-throughput screening of
small-molecule libraries, together with medicinal chemistry efforts, led to the identification of additional Hh pathway inhibitors with enhanced pharmacologic properties. A number of these Hh antagonists are currently under investigation in clinical trials (11; see also Fig. 1).

Targeting the Hh Pathway Induced a Remarkable but Transient Clinical Response in a Metastatic Medulloblastoma Patient

Vismodegib, a potent synthetic oral SMO inhibitor that was generated through intensive medicinal chemistry efforts, is one such compound that is currently under clinical evaluation (12). Vismodegib treatment of a medulloblastoma patient who harbored widespread metastatic disease resulted in a rapid and dramatic tumor regression, seemingly at all tumor sites, which was associated with significant pain reduction and weight gain (3). Analysis of the patient’s primary and metastatic tumors taken prior to treatment revealed a somatic mutation in *PTCH1* (*PTCH1*–W844C), as well as upregulated expression of Hh target genes, supporting the premise that the disease was driven by hyperactivation of the Hh pathway. Despite the robust initial response, positron emission tomography scans taken ~3 months after initiation of treatment showed evidence of tumor regrowth at multiple sites. The patient was removed from the phase 1 study, and the disease then progressed quickly despite a series of subsequent therapies (3). This case study highlights the significant therapeutic advantage that may be gained by targeting the Hh pathway in appropriate contexts, but also brings to light the harsh reality of acquired resistance to SMO-targeted therapies.
To explore the mechanisms of acquired resistance to vismodegib, Yauch and colleagues (13) analyzed a sample from a progressing lesion taken from the medulloblastoma patient to assess the status of known components of the Hh pathway. Sequencing of *PTCH1* confirmed the presence of the previously detected *PTCH1*-W844C mutation, which was accompanied by loss of heterozygosity. Intriguingly, in addition to the *PTCH1* mutation, they identified a *SMO* mutation, a heterozygous G-to-C missense mutation at position 1697 that is predicted to change codon 473 from Asp to His (D473H). *SMO-D473H* was not detected in the pretreatment biopsies, although it is possible that the mutation was present at a frequency below detection levels. Alternatively, it may have arisen at a tumor site different from the one initially biopsied and reseeded all of the metastatic sites following the initial response to vismodegib. *In vitro* analysis of SMO-D473H indicated that this mutant is competent to transduce the Hh signal and is likely not inherently oncogenic; like SMO-WT, its activity is blocked by expression of *PTCH1* (13). However, further investigation showed that, unlike SMO-WT, SMO-D473H is refractory to inhibition by vismodegib. Of note, loss of sensitivity of SMO-D473H to vismodegib was accompanied by a loss of physical interaction between the drug and SMO (13). This mechanism of drug resistance (i.e., a deficiency in drug-target binding conferred by a single amino acid change) is analogous to the frequently detected BCR-ABL T315I sub-substitution that is responsible for imatinib resistance in chronic myeloid leukemia (CML) patients (14).

**Second-Generation Antagonists May Combat Resistance Driven by SMO mutations**

One approach to overcoming resistance generated by specific mutations in the drug target is to develop second-generation inhibitors that retain activity in the presence of the resistance mutation. This strategy has been used with some success in the case of both EGFR and BCR-ABL resistance mutations; for example, nilotinib, a second-generation inhibitor of the ABL kinase, was shown to have clinical activity in imatinib-resistant CML patients (15). An effort to identify second-generation SMO antagonists that display activity in vismodegib-resistant tumors revealed that HhAntag (a benzimidazole) exhibited a similar degree of efficacy in its inhibition of SMO-WT and SMO-D473H (16). In addition, a member of the bis-amide class of SMO inhibitors (compound 5) showed activity against vismodegib-resistant SMO in both cell-based assays and a vismodegib-resistant mouse model (described below), supporting the possibility of developing second-generation inhibitors of mutant SMO (16).

An attractive alternative is the use of antagonists with a mechanism of action clearly distinct from that of vismodegib. Itraconazole, a systemic azole antifungal agent that targets cytochrome P450, was recently shown to inhibit Hh signaling, although it is significantly less potent than the pathway inhibitors currently in clinical development (17). Itraconazole purportedly acts on SMO by a mechanism distinct from that of cyclopamine, although exactly how it functions remains to be determined. Considering this distinct mode of binding, it is possible that itraconazole will retain its efficacy against vismodegib-resistant SMO mutants. The use of antagonists that target the Hh pathway downstream of SMO, such as GANT61, which blocks GLI function, is likewise an appealing therapeutic strategy in the context of resistance driven by SMO mutations (18).

**Mouse Models of Hh Pathway Inhibitor Resistance Faithfully Recapitulate the Scenario Seen in the Medulloblastoma Patient, and Point to Alternative Mechanisms of Resistance Downstream of SMO**

Mouse models have proved to be useful for exploring other potential mechanisms of resistance to SMO antagonists. In one study (13), medulloblastoma tumors arising in *Pch<sup>+/−</sup>*/*p53<sup>−/−</sup> mice were implanted s.c. into nude mice and intermittently challenged with vismodegib until the tumors stopped responding to twice-daily dosing. In a separate study (19), a similar approach was used to generate allograft mouse models resistant to NVP-LDE225, a potent and selective oral SMO antagonist from a newly described structural class. Intermittent (suboptimal) dosing of vismodegib was required to generate resistant tumors that emerged ~45 days after the initial dose. In contrast, continuous dosing of NVP-LDE225 (at varying drug concentrations and dosing schedules) resulted in resistant tumors after only 13 days of treatment. It is surprising that drugs of apparently similar potency should promote the emergence of resistance at such different frequencies in models that were developed on the same *Pch<sup>+/−</sup>*/*p53<sup>−/−</sup> platform, and this finding suggests that subtle differences in genetic background or drug behavior may have an impact on the initiation of resistance to SMO inhibitors.

As the molecule driving resistance in the medulloblastoma patient, *Smo* was sequenced in all drug-resistant tumor lines. Particularly striking was the discovery of a heterozygous A-to-G missense mutation at position 1944 in one of the vismodegib-resistant lines (13). This single nucleotide switch results in an Asp-to-Gly change at residue 477 (D477G), which corresponds exactly to the human D473 residue mutated in the medulloblastoma patient. Moreover, an *in vitro* analysis of SMO showed that substitution of D473 with any other amino acid allowed SMO to retain functionality (apart from the possibly misfolded D473P mutation) but reduced sensitivity to vismodegib, which correlated to a reduction in drug-target binding (16). These data show that, although SMO-D473 is not essential for SMO activity, it is critical for vismodegib binding (16). Of the 135 NVP-LDE225-resistant lines generated, 7 contained missense mutations in *Smo*, representing substitutions at 5 different residues (19), none of which was D477 (the mouse equivalent of human D473). However, SMO-D473H significantly impairs the ability of NVP-LDE225 to inhibit Hh signaling *in vitro* (G. Dijkgraaf, unpublished data). It thus appears that mutations at multiple sites in SMO can confer resistance to SMO antagonists. The question remains as to...
whether any of these residues participate directly in drug binding, or if mutation at these sites induces a conformational change that indirectly affects drug binding.

Upregulation of Gli2, a downstream effector of the Hh pathway, was identified as an important alternative mechanism that confers resistance against SMO antagonists. Amplifications of chromosomal regions containing Gli2 were discovered in a model of vismodegib resistance (16) and in 2 of 3 NVP-LDE225-resistant lines analyzed by comparative genomic hybridization (19). Knockdown of Gli2 in cells isolated from Gli2-amplified NVP-LDE225-resistant tumors resulted in partial reconstitution of sensitivity to the inhibitor (19). The high frequency of Gli2 amplification in NVP-LDE225-resistant tumors (50% of Ptch+/− p53−/− tumors analyzed by quantitative real-time PCR) may also explain, at least in part, the observed high penetrance of Hh pathway reactivation in resistant tumors. The reactivation of the Hh pathway downstream of PTC1 and SMO is in keeping with the notion of oncogenic addiction, i.e., in this case, the Hh pathway is critical for the oncogenic pathology of medulloblastoma. Indeed, an additional vismodegib-resistant line displayed an amplification of the Hh target gene, ccnd1, which was previously identified as a key element in the pathogenesis of medulloblastoma in Ptc+−/− mice (16, 20).

The PI3K Pathway Is Implicated in the Pathogenesis and Resistance of Hh-Driven Medulloblastoma in Mouse Models

Surprisingly, IGF1R-PI3K target genes were much enriched in NVP-LDE225-resistant samples when compared with drug-sensitive samples, suggesting that a compensatory upregulation of this pathway may contribute to the development of resistance (19). This mechanism was observed in the case of erlotinib/gefitinib resistance (14). In addition, a combination of NVP-LDE225 and the PI3K class 1 inhibitor NVP-BKM120, when coadministered from the time of initial treatment, appeared to delay the onset of resistance and subsequent tumor regrowth (17). Of importance, though, the treatment of established NVP-LDE225-resistant tumors with NVP-BKM120 did not have a significant impact on tumor regrowth. It may be that PI3K pathway activation in NVP-LDE225-resistant tumors is driven downstream of PI3K itself, explaining the lack of efficacy of the PI3K inhibitor in established NVP-LDE225-resistant tumors. Although PI3K target genes were enriched in a number of resistant versus sensitive tumors in the NVP-LDE225 study (19), no such enrichment was seen in the vismodegib study (16). It is possible that the small number of vismodegib-resistant models analyzed was simply insufficient to uncover PI3K pathway upregulation as a potential mechanism of resistance. Although NVP-BKM120 administered as a single agent failed to affect tumor development (16), treatment of both vismodegib-sensitive and vismodegib-resistant tumors with the PI3K inhibitor GDC-0941 significantly delayed tumor growth (14). This finding, together with the delayed onset of resistance in the presence of combined NVP-LDE225 and NVP-BKM120, strongly argues that targeting the PI3K pathway in Hh-dependent tumors is an avenue well worth pursuing.

Conclusions

The dramatic clinical response of a medulloblastoma patient to vismodegib emphasizes the great therapeutic potential of targeting the Hh pathway in specific cancers. However, the rapid reemergence of disease in that patient indicates that, like other targeted therapies, acquired resistance to Hh pathway inhibitors is a potential hurdle to durable response in the clinic. In the context of resistance to kinase inhibitors, elucidation of mechanisms of resistance has contributed to the clinical success of second-generation inhibitors. Understanding how resistance against SMO inhibitors might occur in the clinic is thus a top priority. In this regard, patient samples will clearly be extremely valuable; however, the striking recapitulation of the patient-derived D473H mutation in the vismodegib-resistant mouse model highlights the value of exploring mechanisms of drug resistance in other systems. The studies described here provide an impetus for continuing research into 2 distinct approaches to circumvent resistance against SMO antagonists, both in medulloblastoma and in other settings: second-generation inhibitors and targeting of the P3K pathway. Although the emergence of resistance to a SMO antagonist has only been documented in a single case of medulloblastoma, the potential emergence of resistance should be carefully monitored in the ongoing clinical trials of Hh pathway inhibitors in basal cell carcinoma and other cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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