MEK1/2 as a Therapeutic Target in Sickle Cell Disease

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Abstract

Identification of novel therapeutic targets has improved diagnostics and treatment of many diseases. Many innovative treatment strategies have been developed based on the newly identified biomarkers and key molecules. Most of the research focused on ways to manipulate signaling pathways by activating or suppressing them, validate new therapeutic targets for treatment, and epigenetic treatment of diseases. With the identification of aberrations in multiple growth pathways, the focus then shifted to the small molecules involved in these pathways for targeted therapy. In this communication/short review, we highlight the importance of identification of abnormal activation of the mitogen-activated protein kinase (MAPK), ERK1/2, and its upstream mediator MEK1/2, in erythrocytes in patients with sickle cell disease (SCD) critical for the adhesive interactions of these cells with the endothelium, and leukocytes promoting circulatory obstruction leading to tissue ischemia and infraction. We also discuss how targeting this signaling cascade with MEK1/2 inhibitors can reverse acute vasoocclusive crises in SCD.

Keywords
Sickle cell disease; Sickle erythrocytes; Leukocytes; MEK1/2 inhibitors; Adhesion; Endothelium

Introduction

In sickle cell disease (SCD), prevention and treatment of acute vasoocclusive crises remain a therapeutic challenge. SCD is a monogenetic disorder due to a single mutation that occurs in the globin b-chain, resulting in the formation of hemoglobin S (HbS). Recurrent vasoocclusive episodes (VOCs) contribute to morbidity and accelerated mortality, due primarily to acute pain crises and progressive irreversible ischemic end-organ damage. The pathophysiology of vasoocclusion is complex and multifactorial, involving deoxygenation-dependent HbS polymerization and sickling leading to impaired rheology [1–4], altered cell adhesion [5–11], activation of leukocytes [9,12], the endothelium [13–17] and the coagulation cascade [18–20], oxidative stress [21–26], and both intracellular and...
extracellular signaling pathways [27–33]. While these factors may act in a positive feedback-loop to drive the pathophysiology of SCD, the gravity and the impact of each factor in promoting VOCs appears to vary. Recent studies have shown that deoxygenation of Hbs-dependent rheologic alterations are suitable by themselves to significantly increase viscosity, slowing blood flow velocity at arterial oxygen tension even in the absence of inflammation, adhesion, and endothelial and leukocyte activation [3]. Multiple anti-sickling agents are currently being tested in clinical trials. The most promising ones include, SCD-101, a botanical-derived drug similar to niprisan [34]. In a phase 1B dose-escalation study of 26 HbSS and HbS/β0-thalassemia patients, SCD-101 was well tolerated and, at higher doses, it alleviated chronic pain and fatigue, yet, this agent had no impact on Hb levels [35]. Aes-103 (5-hydroxymethyl-furfural) is also an anti-sickling agent undergoing clinical trials that is known to directly modify Hb structure [36,37]. More recently, GBT440 (a polyaromatic aldehyde) has been developed, which enhances oxygen affinity similar to Aes-103, but with higher specificity and at much lower concentrations [38]. A dose-dependent increase in Hb levels within 2 weeks of initiating therapy of GBT440 was accompanied by a prolonged red cell life span in SCD patients [39,40]. The efficacy and safety of the drug is being investigated in a double-blind multicenter phase 3 study.

Although targeting HbS polymerization might be a promising strategy to prevent acute pain crises, more recently, in vitro studies under hypoxic conditions have demonstrated the simultaneous and synergistic effects of adhesion and polymerization of deoxygenated HbS in human erythrocytes containing primarily HbSS (SS RBCs) on the mechanisms underlying vasoocclusive pain crisis [10]. However, abnormal adherence of SS RBCs with the endothelium can occur even under normoxic conditions and for different RBC shapes [41], and under various flow conditions [42]. Therefore, adhesion of SS RBCs to endothelium has been postulated to be a major candidate in the initiation of vasoocclusion [41,43,44]. In fact, more than two decades ago, Hebbel and colleagues have made critical observations: they showed that SS RBCs were abnormally adherent to cultured endothelial cells, and the degree of adhesivity shown by individual patients’ RBCs correlated with their clinical disease severity [41]. We now know that SS RBCs express numerous adhesion molecules. Many erythrocyte adhesion receptors on SS RBCs have been shown in either in vitro or in/ex vivo studies to mediate adhesion of these sickle cells to either extracellular matrix (ECM), endothelial cell (EC), leukocyte or other cellular ligands [9,29,30,32,33,45–48]. Additionally, when SS RBCs bind to leukocytes, they cause leukocyte activation and adhesion [9]. Once leukocytes adhere to endothelial cells, they also contribute to vasoocclusion by recruiting SS RBCs to adhere to stationary leukocytes and further blockading small vessels [11]. In contrast, although normal RBCs express all the adhesion receptors expressed by mature SS RBCs, they exhibit little adhesive phenotype [29,46,48–51]. The fact that SS RBCs express a higher level of some of these receptors, such as the BCAM/Lu receptor [52], than do normal RBCs, might in part explain the adhesivity of SS RBCs. However, the degree to which receptors may be overexpressed on SS RBCs is relatively small compared to the degree to which adhesion is enhanced [52]. In addition, while SS reticulocytes express higher levels of CD36 and a4b1 integrin than do normal reticulocytes, in many experimental systems it is primarily the more mature dense sickle cells rather than the SS reticulocytes that are most adherent [53]. Several studies have
attempted to prevent cell adhesion using different strategies, since the rationale of targeting adhesion is very strong. For instance, targeting selectins with a pan-selectin inhibitor, rivipansel (GMI-1070) \[54\], seems to shorten the duration of acute sickle pain crises accompanied by a marked reduction in opioid use in a phase II trial \[55\]. Crizanlizumab (SEG101), a monoclonal antibody against P-selectin, was also efficient in lowering the rate of sickle cell-related pain crises associated with a low incidence of adverse events in a randomized double-blind study \[56\]. Nonetheless, the efficacy of these two agents does not appear to be superior to hydroxyurea, the only FDA-approved drug for SCD. Hydroxyurea can act as both an anti-sickling, since this drug can elevates fetal Hb (HbF) levels, but only in some SCD patients \[57,58\], and anti-adhesive agent \[58,59\]. Despite hydroxyurea reduces the frequency of vaso-occlusive episodes \[60\], patients with SCD remain to be treated primarily with analgesics for pain relief. Lack of real effectiveness by these anti-adhesive drugs in treating VOCs when used alone, is possibly due to the fact that these agents are aimed to target the proximate, but not the route, cause of events that precipitate vasoocclusion.

Among the critical remaining questions regarding adhesion of SS RBCs to their ligands that are not fully answered, are: 1) What makes SS RBCs adherent? and 2) What makes SS RBCs capable of activating leukocytes? Our investigations have focused on signaling mechanisms that enhance SS RBC adhesion and interaction with other cells and ECM ligands, and SS RBC-induced activation of other cells. Just not long ago, we begun to understand why these adhesion receptors are more active on SS RBCs. Investigations by us and others led to the discovery that SS RBCs possess kinases that remained activated to mediate abnormal adherence of SS RBCs via phosphorylation/activation of RBC adhesion receptors, including LW (ICAM-4, CD242) blood group glycoprotein \[29,31,32\], CD44 \[27,32\], BCAM/Lutherin (Lu) \[46,61–63\], and a4b1 integrin \[64\]. So far, the identified kinases that remained active or hyper-activated in both immature and mature SS RBCs critical for adhesion of these sickle cells, are kinases of the b2-adrenergic receptor and the oxidative pathways, including the mitogen-activated protein kinases (MAPKs) known as the extracellular signal regulated kinases ERK1 and ERK2, protein kinase A (PKA), G protein-coupled receptor kinase 2 (GRK2), and the tyrosine kinases p72Syk and p60-c-Src \[27,29,31,32,46,61–63\]. The ERK1/2 pathway is a unique and critical signaling mechanism in SCD. ERK1/2 was found aberrantly active in SS RBCs, and present but inactive in normal RBCs \[31\]. Aberration in ERK1/2 activation occurred in both immature and mature human SS RBCs, but could not be detected in normal human reticulocytes \[31,65\]. In vitro and in vivo studies have revealed that abnormal human SS RBC ERK1/2 activation mediates adhesion of these sickle cells to the vascular endothelium \[66,67\]. Further studies have indicated that human SS RBC interaction with naïve neutrophils activates adhesion of naïve neutrophils to endothelial cells; this effect is also stimulated by the RBC MEK1/2 and its downstream ERK1/2 pathway \[66\]. More recently, we evidenced that ERK1/2 activation can be up-regulated in SS RBCs, but not in normal RBCs, by hypoxia/prolonged reoxygenation \[27\]. Hypoxia/prolonged reoxygenation-stimulated ERK1/2 activation creates an intra-erythrocytic positive feedback loop with reactive oxygen species (ROS) generated by NADPH oxidases, and GRK2 to mediate SS RBC adhesion to endothelium in vitro, and vasoocclusion in a mouse model of vasoocclusion in vivo \[27\]. Excessive ROS produced by
HbS autoxidation within SSRBCs likely participate in the maintenance and/or increased ERK1/2 and GRK2 activities and the downstream mechanisms of sickle cell adhesion.

The MAPK cascade is a critical signal transduction pathway involved in regulation of cellular activities including proliferation [68–70], growth, differentiation [71,72], apoptosis [69,70], and angiogenesis [73,74]. Protein kinases in these cascading pathways transduce extracellular signals into intracellular responses through a series of protein-protein interactions and phosphorylation. Recurrent mutations are noted in the receptor tyrosine kinases that activate this pathway [75]. In 30% of human malignancies, these signal transduction pathways are unusually activated [76]. Constitutive activation of the pathway leads to tumorigenesis, uncontrolled cell proliferation, and resistance to apoptosis [77]. Seven distinct mitogen-activated protein kinase kinase (MEK) proteins are involved in the sequential phosphorylation, upstream of the transduction pathway, and a number of target molecules have been identified. MEK1 and MEK2 are highly specific kinases that phosphorylate serine/threonine and tyrosine residues of ERK1 and ERK2. ERK1/2 are the only known substrates of MEK1/2, and are aberrantly activated in tumor cell lines. Solid tumors like melanoma, lung carcinoma, and colon carcinoma also show increased activation of ERKs [78,79], making them potential therapeutic targets for treatment. Being ATP-uncompetitive, inhibition of MEKs does not affect other kinases and thus prevents unwanted side effects.

Data in SCD now reveal a potential therapeutic benefit of the two MEK1/2 inhibitors RDEA119/BAY 869766, tested in a phase 1 clinical study in cancer, and trametinib, an FDA-approved drug for melanoma treatment. MEK1/2 inhibitors prevented the inception and/or progression of vasoocclusion in vivo in two animal models of vasoocclusion [65]. In the earlier studies, we show that the inhibitors were able to prevent the adhesion of human SS RBCs in the venules in mouse models of vasoocclusion [66,67]. Later on, we show that RDEA119 and trametinib given intravenously or orally were effective in reversing the adhesion of both SS RBCs and leukocytes, and established vasoocclusion in two SCD murine models of vasoocclusion, thus enabling the restoration of blood flow in the vessels [65]. The reversal effect of MEK1/2 inhibitors on SS RBC and leukocyte adhesion in SCD mouse models was rapid and efficient [65]. Moreover, these inhibitory effects did not require high doses of drug. These compounds reversed SS RBC and leukocyte adhesion at very low doses (0.025, 0.05 and 0.1 mg/kg) and within the first 10–30 min of drug administration; leukocyte adhesion further decreased over time [65]. Interestingly, adhesion of SS RBCs and leukocytes was reversed by the cleavage of endothelial E-selectin, P-selectin and avb3 integrin, and leukocyte CD44 and b2 integrin [65]. Our data were in agreement with previous studies showing that the MEK1/2 inhibitor PD98059 considerably reduced endothelin-1-activated neutrophil adhesion [80]. Inhibition of ERK1/2 in activated neutrophils down-regulated the expression of adhesion molecules [80]. The MEK1/2 inhibitor, U0126, was also able to attenuate cisplatin-induced renal injury by decreasing inflammation and apoptosis in mice [81].

Although the sequence of events leading to suddenly symptomatic vasoocclusion in humans with SCD is still poorly understood, the fact that MEK1/2 inhibitor therapy reverses both RBC and leukocyte adhesion triggered by inflammation in a severe sickle mouse model and

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an adoptive nude mouse model of vasoocclusion [65–67], portend for the potential clinical relevance of such intervention. Our data also argue that existing MEK1/2 inhibitors may be safe compounds if used acutely or for a short-term in SCD patients to treat vasoocclusion, since daily administration for 14 days of trametinib at 0.3 mg/kg, a dose 12-fold higher than the lowest effective dose of RDEA119 (0.025 mg/kg), did not exacerbate anemia or caused thrombocytopenia in sickle mice [65]. Additionally, shorter-term use (7 days or less) of these drugs during acute pain crises in SCD should minimize any drug-associated side-effects [65]. Thus, despite the complexity of the pathophysiology of VOCs, MEK1/2 inhibitors may be an effective therapy in SCD, by affecting not only SS RBCs, but also leukocytes and possibly the endothelium [65–67,80,81].

Some of these MEK proteins are being evaluated as therapeutic targets in cancer clinical studies for the past few years. CI-1040 was the first MEK1/2 inhibitor to be used in a clinical setting and was evaluated in the treatment of non-small cell lung cancer (NSCLC), colon, pancreatic and breast cancers [82]. Although the molecule showed promising results in phase 1 with 73% inhibition of phosphorylation, it failed to have encouraging clinical activity in the phase 2 studies [82]. PD0325901, an improved version of CI-1040 with better bioavailability and potency, also failed to meet the primary endpoints in phase 2 clinical trial [83]. Selumetinib was studied in different solid tumors and hematologic cancers in multiple phase 2 clinical studies [84,85]. The antitumor activity of the molecule was not significant in most of the malignancies [84,85]. Partially good responses were observed with the MEK1/2 inhibitor GDC-0973 (XL-518, RG7421) in BRAF-mutated melanoma and BRAF-mutated pancreatic cancer [86,87]. Trametinib attained 10% objective responses, particularly in patients with BRAF-mutant melanoma [88]. This MEK1/2 inhibitor was then advanced to phase 3 study where a median progression free survival of 4.8 months was reported [89]. Currently, trametinib is the only FDA-approved drug for treatment of melanoma patients. Other MEK1/2 inhibitors like pimasertib (MEK162) [90–95], AZD8330 [96–98], and RO5126766 [99–102] are under study, but none has demonstrated good therapeutic response of interest. One of the reasons for the failure might be that mutated proteins, like RAS and RAF, may have alternative pathways that escape the inhibitory effect of the drugs. Further, if the cancer is produced by mutations in multiple growth pathways, inhibition of one pathway may not be enough for cytotoxicity or inhibition of proliferation. In the present clinical studies, single-agent MEK1/2 inhibitor activity is shown in BRAF-mutant melanoma, but stronger responses are reported when it is used in combination with BRAF inhibitors [103].

In summary, clinical and laboratory studies validate the use of these molecular targets in the development of effective therapeutic agents for diseases like SCD, where we showed that MEK1/2 inhibitors at doses at least 20-times less than the doses tested in animal models to treat cancer, revealed to be effective and safe in reversing an acute vasoocclusive crisis in vivo [55]. Although many studies have been completed and some are ongoing, the clinical relevance of these molecules indicates that we are moving in the right direction.
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List of Abbreviations

- SCD: Sickle Cell Disease
- MAPK: Mitogen-Activated Protein Kinase
- HbS: Hemoglobin S
- VOCs: Recurrent Vasocclusive Episodes
- HbF: Fetal Hemoglobin
- SS RBCs: Sickle Erythrocytes
- ECM: Extracellular Matrix
- EC: Endothelial Cell
- ERK1/2: Extracellular Signal Regulated Kinases
- MEK: Mitogen-Activated Protein Kinase Kinase
- PKA: Protein Kinase A
- GRK2: G Protein-Coupled Receptor Kinase 2
- ROS: Reactive Oxygen Species
- NSCLC: Non-Small Cell Lung Cancer

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Table 1:
List of promising compounds in clinical trials and FDA-approved for treatment of SCD and/or cancer.

| Compound Name | Target     | Function                  | Outcomes                                           | Phase of Clinical Trial | Disease                               |
|---------------|------------|---------------------------|---------------------------------------------------|-------------------------|---------------------------------------|
| Hydroxyurea   | b-globin gene | Up-regulates HbF expression | Reduces the frequency of vasoocclusive episodes   | FDA-approved            | SCD                                   |
| SCD-101       | HbS        | Anti-sickling             | Alleviates Chronic pain and fatigue               | Phase 1b                | SCD                                   |
| Aes-103       | HbS        | Anti-sickling             | Modified Hb structure                             | Phase 1                 | SCD                                   |
| GBT440        | HbS        | Anti-sickling             | Enhances HbS oxygen affinity                      | Double-Blind phase 3    | SCD                                   |
| Rivipansel(GMI-1070) | Selectins | Pan-selectin inhibitor   | Reduces Duration of acute pain crises, and opioid use | Randomized double-Blind phase 3 | SCD                                   |
| Crizanlizumab(SEG101) | P-selectin | Anti-P selectin monoclonal antibody | Lowers pain crises                               | Phase 2                | SCD                                   |
| RDEA119/BAY869766 | MEK1/2    | MEK1/2 inhibitor          | Well-tolerated with some clinical benefit across a range of tumor types | Phase 1                 | Advanced metastatic solid tumor       |
| Trametinib    | MEK1/2     | MEK1/2 inhibitor          | Melanoma treatment                                | FDA-approved            | BRAF-mutant melanoma                  |
| CI-1040       | MEK1/2     | MEK1/2 inhibitor          | No clinical activity                              | Phase 2                 | NSCLC, colon pancreatic, and breast cancers |
| PD0325901     | MEK1/2     | MEK1/2 inhibitor          | No clinical activity                              | Phase 2 (terminated)    | Advanced breast and colon cancer, and melanoma |
| Selumetinib(AZD6244) | MEK1/2    | MEK1/2 inhibitor          | No significant antitumor activity                 | Phase 2 (terminated)    | Breast cancer                         |
| GDC-0973 (XL-518, RG7421) | MEK1/2    | MEK1/2 inhibitor          | Partially good responses                          | Phase 2                 | BRAF-mutated both melanoma and pancreatic cancer, and breast cancer |
| Pimasertib(MEK162) | MEK1/2    | MEK1/2 inhibitor          |                                                  | Phase 2                 | N-Ras-mutated cutaneous melanoma, and ovarian cancer |
| AZD8330       | MEK1/2     | MEK1/2 inhibitor          |                                                  | Phase 1                 | Advanced malignancies                 |
| RO5126766     | MEK1/2     | MEK1/2 inhibitor          |                                                  | Phase 1                 | Advanced NSCLC                        |

Drug’s named and their targets, function, clinical benefits, and their phase in clinical trial for SCD and/or cancer treatments are presented.