# Discoidin Domain Receptor 2 Signaling Networks and Therapy in Lung Cancer

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**Abstract:** Discoidin domain receptor 2 (DDR2) is an atypical receptor tyrosine kinase that binds to and is activated by collagen in the extracellular matrix. Recent exon sequencing studies have identified *DDR2* to be mutated with a 3% to 4% incidence in squamous cell cancers of the lung. This article summarizes the current state of knowledge of DDR2 biology and signaling in lung squamous cell cancer. It also explores the context-dependent role of this receptor as both an oncogene and a tumor suppressor in cancer cells. Promising therapeutic opportunities based on existing and novel targeted small molecule inhibitors against DDR2 may provide new strategies for treating lung squamous cell cancer patients.

**Key Words:** Discoidin domain receptors, Lung cancer, Signal transduction, Collagen, Kinase inhibitors

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Squamous cell cancers (SCCs) of the lung develop from bronchial epithelial cells as a result of squamous metaplasia and are typically found in smokers.[1] The standard of care for this disease is a chemotherapy regimen of four to six cycles of platinum doublets in the first-line setting.[2] Prognosis is often poor with objective response rates of 30% to 40% and a median survival of 12 months for patients with stage IIIb/IV disease.[3] Clinical trials of targeted therapies in lung SCCs have not shown any patient benefit and in some cases led to greater toxicity. For instance, treatment with the vascular endothelial growth factor receptor inhibitor bevacizumab resulted in an increased risk of bleeding complications in SCC patients in phase II trials and is currently approved only in non-squamous non–small-cell lung carcinoma.[4,5] Another example is the insulin-like growth factor-1 receptor inhibitor figitumumab that showed increased toxicity when combined with chemotherapy compared with chemotherapy alone, resulting in the closure of phase III clinical trials.[6] The failure of these trials underscore the need for a comprehensive understanding of the biology of lung SCC, in particular how genetic aberrations manifest at the signaling level to promote tumorigenesis and dictate therapeutic response. The differential responses to targeted therapies between lung adenocarcinomas and SCCs in the clinical setting suggest that the biology and genetic landscape of these two diseases are unique. Indeed, recent lung SCC sequencing studies by Hammerman et al.[7,8] demonstrate that the genomic aberrations in SCCs are distinct from adenocarcinomas.

## DDR2 AND LUNG SCCs

In an exon sequencing study, Hammerman et al.[8] identified the discoidin domain receptor 2 (*DDR2*) gene as a potential oncogenic target in lung SCC. The authors screened 290 tumors and cell lines and reported a 3.8% incidence of *DDR2* point mutations in lung SCC samples. This frequency is similar to the incidence of EML4-ALK in lung adenocarcinomas.[9] Additional *DDR2* mutations at 4.4% frequency have since been identified in an independent cohort of lung SCC patients.[10] It is likely that early targeted sequencing studies failed to identify any aberrations in the *DDR2* gene because of small patient cohort size.[11,12] A lower mutation frequency of 1.1% was reported in a large-scale next generation sequencing study in a cohort of 178 SCC patients performed by the TCGA Network,[13] whereas no mutations were found in a screen of 166 SCC biopsies from Japanese patients.[14] In the latter case, Sasaki et al[14] proposed that their inability to detect any *DDR2* mutations may have been related to ethnic differences in the sample populations.

The *DDR2* point mutations are not localized to hotspot regions and are distributed throughout the gene, including the extracellular ligand-binding discoidin domain and the cytoplasmic kinase domain. Interestingly, data emerging from lung adenocarcinoma sequencing studies have also identified *DDR2* mutations at 2% to 5% frequency (http://www.cbioportal.org/).[15] Again, these mutations are spread across the gene but were not found to be significantly enriched over the background mutational rate of the tumors analyzed. The biological role of DDR2 in lung adenocarcinoma remains to be investigated. DDR2 is a receptor tyrosine kinase, which also functions as an adhesion receptor that is activated by collagen, a major component of the extracellular matrix in the lung.[16] Hammerman et al.[8] showed that a subset of these DDR2 mutants is tumor promoting in cell lines in vitro. Depletion

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of mutant DDR2 using RNA interference in lung SCC cells demonstrated oncogene addiction. Importantly, this class of mutations is sensitive to inhibition by the FDA-approved tyrosine kinase inhibitor dasatinib in both in vitro assays and in subcutaneous xenograft models in vivo, making it clinically actionable and amenable to rapid advancement into lung SCC trials.[8,17]

There have been two reports of tumor shrinkage in response to dasatinib treatment in lung SCC patients harboring the DDR2 kinase domain S768R mutation.[8,18] In the first case described by Hammerman et al.[8] a combination of erlotinib (an epithelial growth factor receptor inhibitor) and dasatinib was administered to a patient whose disease had progressed despite carboplatin and paclitaxel therapy. Within 2 months of treatment, this patient showed a partial response and tumor shrinkage. She remained on the combination regimen for 14 months before discontinuation because of toxicity. In the second case study reported by Pitini et al.,[18] the patient had a rare instance of a BCR-ABL positive chronic myelogenous leukemia and a DDR2 S768R mutation in a lung SCC lesion. Dasatinib therapy resolved both the chronic myelogenous leukemia and the lung tumor after 10 weeks and the patient remained clinically well 8 months into the treatment. These case studies provide an indication that a subset of DDR2 mutations is oncogenic in this disease. Ongoing phase II trials assessing the efficacy of dasatinib in lung SCC (NCT01491633 and NCT01514864) are undertaking correlation analysis of DDR2 mutational status with response to therapy to validate the clinical relevance of these experimental findings.[19]

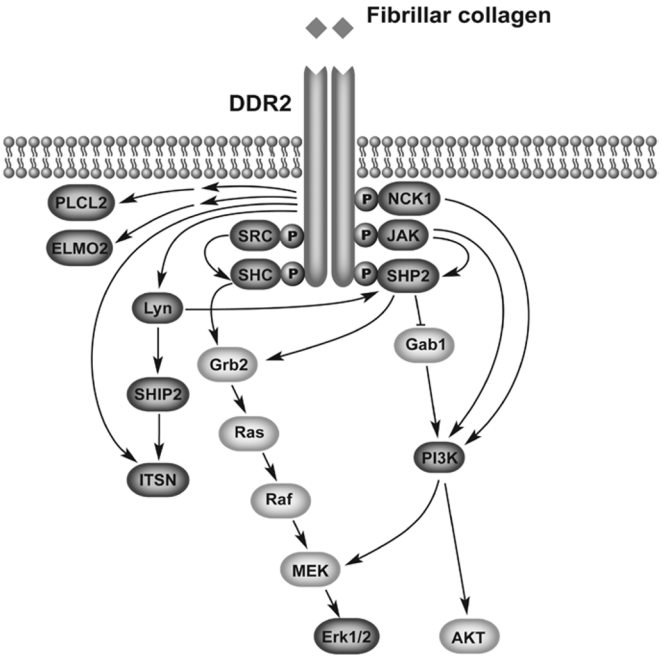


**FIGURE 1.** Depiction of signaling pathways activated downstream of Discoidin domain receptor 2 (DDR2). Binding of collagen to the extracellular domain of DDR2 triggers the autophosphorylation of its cytoplasmic domain. This results in the recruitment of downstream adaptor proteins, kinases, and phosphatases including SHC, NCK1, SRC, and SHP-2. As a consequence, a series of canonical signaling pathways are initiated including the ERK1/2 and PI3K cascades.

## DDR2 SIGNALING NETWORKS

DDR2 has been implicated in a number of cancer types and has been shown to play a role in driving proliferation and metastasis (see Borza and Pozzi [20] and Valiathan et al.[21] for recent excellent reviews on this topic). There is limited information available on the signaling pathways activated by DDR2 on collagen engagement. Work done by several research groups have shown that DDR2 activates important signaling components including SHC, SRC, JAK, ERK1/2, and PI3K (summarized in Fig. 1).[16] In addition, Hammerman et al.[8] used phosphorylation of STAT5 as a biological readout for DDR2 activity, although this signaling protein is unlikely to be a bona fide downstream substrate of the DDR2 pathway but rather the result of a survival signaling cascade in the IL-3 dependent Ba/F3 murine cell line. DDR2 also exhibits crosstalk with other cell surface receptors such as the integrins and RTKs resulting in diversification of downstream signal transduction networks.[22–25] Our laboratory has recently performed a global phosphoproteomic screen of DDR2 signaling activated by collagen I and identified 45 signaling effectors downstream of this receptor.[26] In addition to the previously identified signaling nodes ERK1 and PI3K, these effectors also include novel protein substrates such as Lyn, SHP-2, SHIP-2, and PLCL2 (Fig. 1). We further show that these signaling events are independent of integrin activation by collagen and are specific to the DDR2 pathway. Similar to previous reports of signal transduction pathway adaptation in the epithelial growth factor receptor mutants often found in lung adenocarcinoma,[27,28] the identification of DDR2-specific signaling nodes will facilitate future studies on network reprogramming events that occur on acquisition of DDR2 mutations in cancer cells.

## IS DDR2 AN ONCOGENE OR A TUMOR SUPPRESSOR?

There is some controversy regarding the role of DDR2 in cancer. Although Hammerman et al.[8] showed that a subset of the DDR2 mutants, including the extracellular discoidin domain variant L63V and kinase domain variant I638F, are oncogenic, these assays were performed in the absence of its physiological ligand collagen. Furthermore, the activation and phosphorylation status of the receptor in these mutants were not established in this study. Fibrillar collagen inhibits cancer cell growth and one mechanism by which this process occurs is through a DDR2-dependent cell cycle arrest in melanoma and fibrosarcoma cells.[29–31] It is plausible that DDR2 functions in a context-dependent manner and in the presence of its natural ligand collagen may act as a tumor suppressor rather than an oncogene. In support of this notion, mRNA levels of DDR2 are diminished in lung cancer tumors compared with matched normal lung tissue, suggesting a potential tumor suppressor role.[11,14] This context dependence is reminiscent of the β1 integrin adhesion receptor that promotes tumor formation in transgenic mouse models of breast cancer but exhibits tumor

 