NIH Public Access
Author Manuscript
Drug News Perspect. Author manuscript; available in PMC 2009 December 31.

Published in final edited form as:
Drug News Perspect. 2008 ; 21(6): 331–335. doi:10.1358/dnp.2008.21.6.1246832.

Disrupting the Rb-Raf-1 Interaction: A potential therapeutic target for cancer

Rebecca K. Davis and **Srikumar Chellappan**[*]
Drug Discovery Program, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612.

Summary

Cell cycle progression in cancer is often mediated by disrupting the function of the retinoblastoma tumor suppressor protein, Rb. One way in which Rb's function is altered is through phosphorylation mediated by cyclin dependent kinases (1). Our studies had shown that the Raf-1 kinase binds and phosphorylates Rb very early in the cell cycle prior to the binding of cyclins and cdks (2). It was also found that human lung cancer tumor samples had increased binding of Raf-1 to Rb, suggesting this interaction could have contributed to the malignancy of these tumors (3). Disrupting the Rb-Raf-1 interaction could inhibit cell proliferation in a multitude of cancer cell lines as well as prevent angiogenesis and tumor growth *in vivo*. Thus, the Rb-Raf-1 interaction is a promising therapeutic target for cancer. This review will highlight the importance of the Rb-Raf-1 interaction in cancer, the search for small molecules capable of disrupting the interaction as well as properties of Rb-Raf-1 disruptors, focusing specifically on RRD-251(Rb-Raf-1 Disruptor 251). This review will also touch on why targeting protein-protein interactions may be a viable alternate better strategy to inhibiting kinase function for cancer therapies.

Keywords

Rb; Raf-1; cell cycle; angiogenesis; xenograft; melanoma

Rb inactivation in Cancer

Perturbations in the Rb pathway are present in almost all cancers, and several mechanisms have been identified for inactivating Rb, in addition to mutation and deletion of the gene itself. Over-expression of cyclin-D or CDK4 kinases from amplification, mutation, or chromosomal translocation can lead to enhanced Rb phosphorylation and poor prognosis (1,4–8). Also, loss or mutation in $p16^{INK4a}$ (cdk inhibitor) can induce excessive CDK4/cyclin D activity and will lead to increased Rb phosphorylation and inactivation. Since p16 is responsible for the control of cyclin D/cdk4 kinase activity, mutations or loss of p16 correlates with Rb activity and are often found in human cancers (9–13). Increased expression of cyclin E/cdk2 or reduced levels of cdk inhibitor $p27^{Kip1}$ also give a poor prognostic factor in many cancers since these too will lead to increased Rb inactivation (1). Another common method for Rb inactivation in human cancers is through the viral oncoprotein E7 (14,15). The tumor-promoting HPV contains at least two genes, E6 and E7, which encode for proteins that interfere with cell-cycle regulation. E7 disrupts the cell cycle via its direct binding to Rb and other members of the retinoblastoma family (p107 and p130). Thus, Rb function is often deregulated in cancer through the binding of viral oncoproteins and hyper phosphorylation by cyclins/CDKs, in addition to being inactivated at the genetic level.

*Corresponding author: Srikumar.Chellappan@moffitt.org, Phone: (813) 745-6892, Fax: (813) 745-6748.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The Rb protein contains at least 18 potential phosphorylation sites; cdk4/6 has been shown to target 4 residues C-terminal to the pocket domain (16,17). Cyclin E-cdk2 complexes have also been shown to modulate Rb function. The Ras/Raf/MEK/MAPK pathway signaling pathway functions in a growth factor dependent manner to upregulate cyclin D1 kinase activity and this in turn regulates Rb phosphorylation and its cell cycle functions (18–20). It has been shown that components of the MAP kinase cascade, including ERK kinases and Raf-1 kinase can phosphorylate Rb in response to proliferative signals (18). Although it has been shown that there is a clear link between growth factor stimulated Ras/MAPK pathway and Rb phosphorylation, other non-growth factors regulating this pathway have also been implicated in modulating Rb function. Studies involving hormones, neurotransmitters and nicotine have also revealed a link between Ras/MAPK signaling and Rb-E2F pathway (3,19).

Rb-Raf-1 Interaction in Cancer

Rb is a nuclear protein and Raf-1 is predominantly cytoplasmic with activation occurring at the plasma membrane. Experiments in yeast two-hybrid assays and *in vitro* binding assays revealed that Raf-1 could bind to Rb (2). The Rb-Raf-1 interaction was signal dependent and Rb-Raf-1 interaction was not detected in quiescent cells. Serum stimulation of quiescent cells induced the binding of Raf-1 to Rb within 30 minutes; this interaction persisted for 2 hours and dissipated before cyclins and cdks bound to Rb (2). Though Raf-1 is predominantly cytoplasmic, a portion of Raf-1 translocates to the nucleus upon serum stimulation where it bound to Rb (2). Raf-1 could efficiently phosphorylate Rb *in vitro*; over-expression of Raf-1 could inactivate Rb and reverse Rb mediated repression of E2F1 mediated transcription as well as S-phase entry (2). The Rb-Raf-1 interaction was found to be mediated by amino acids 10–18 in the N-terminal region of Raf-1 (21). Though Raf-1 bound to the pocket domain of Rb, similar to viral oncoproteins, one major difference is that viral oncoproteins dissociate E2F1 from Rb but Raf-1 does not (21). It is thought that Raf-1 binds to and phosphorylates Rb, priming Rb for further binding and inactivation by cyclins/cdks. Studies have shown that this interaction is dependent on growth factor stimulation; it has also been shown that nicotine and tobacco carcinogens can stimulate the binding of Rb-Raf-1 in whole cells (3).

Given the fact that the Rb-Raf-1 interaction was dependent on mitogenic and nicotinic stimulation, the status of the Rb-Raf-1 interaction in human cancer was examined. Whole-cell lysates were prepared from ten non-small cell lung carcinomas (NSCLC) as well as the adjacent normal tissue that were resected from patients and levels of Rb-Raf-1 interaction was assessed by a immunoprecipitation-western blotting experiment. Rb-Raf-1 interaction was elevated in the tumor tissue compared to the normal adjacent tissue in eight out of the ten pairs examined (3). ChIP assays also revealed a similar result in NSCLC tumor tissues; more Raf-1 was found on the proliferative promoters *cdc6* and *cdc25A* in tumor tissue compared to the normal tissue (3). This suggests that the Rb-Raf-1 interaction might have contributed to the oncogenic process. It can be imagined that smokers as well as cancer patients using nicotine cessation replacement therapy might have elevated Rb-Raf-1 complexes present in their tumors.

Efforts to target Raf-1 signaling pathways and Rb in cancer

It is apparent that mutations leading to Raf activation are the driving force behind many different types of malignancies and there is solid proof of principle for B-Raf and Raf-1 to serve as targets in cancer therapy (22–25). Several attempts have been made to target Raf-1 in pre-clinical as well as clinical trials. Antisense oligonucleotide (ASO) therapy has been attempted by two independent companies to target Raf-1 mRNA. Both ISIS-5132 and leRafAon reached phase I and II clinical trials, yet outcomes were not significant and inhibition of Raf-1 levels were not reached (23). Another attempt at targeting Raf-1 was the small molecule BAY 43-9006 compound that inhibits Raf-1 kinase activity. Further characterization

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

of the bi-aryl urea compound demonstrated inhibition of wild type B-Raf and mutant B-Raf kinase, VEGFR-2, mVEGFR-3, mPDGFR-β, Flt-3, c-KIT, and FGFR-1. BAY 43-9006 inhibits Raf-1 and mVEGFR2 activity with an IC_{50} of 6nM; it's IC_{50}s for B-Raf mut, B-Raft wt, VEGFR2, mVEGFR3, Flt-3, c-kit, p38α, and mPDGFR-β ranges from 12–68nM. BAY43-9006, named Sorafenib or Nexavaar was approved by the FDA for the treatment of renal cell carcinoma (RCC) and liver cancer since previous phase II and phase III results showed significant responses specifically in RCC patients (24,25); it is currently being evaluated for non-squamous non-small cell lung carcinomas. Sorafenib is likely functioning in RCC and liver cancer because of its ability to inhibit many kinases including VEGFR. Competitive inhibitors targeting the ATP-binding site of Raf-1 have also been developed. L-779450 has shown nanomolar activity against Raf-1 over-expressing tumors (26). Targeting Raf-1 kinase activity is an ongoing pursuit to date, and further evaluation of the role of Raf-1 in cancer will reveal the best methods for targeting Raf-1.

Targeting the cell cycle and Rb phosphorylation by inhibiting cyclin-dependent kinases has been studied for well over 15 years (27). Pan-CDK inhibitors such as Flavopiridol and CY-202 have undergone phase II and III clinical trials, yet neither has been approved by the FDA for commercial use (28). Because of their modest activity in the clinic, new generation CDK inhibitors are currently being pursued and evaluated in advanced preclinical settings. Studies from CDK knockout mice have revealed that certain CDKs are not necessary for cell cycle function (29,30). These experiments explain why inhibitors of CDKs are not as efficacious in patients as expected.

Targeting the Rb-Raf-1 interaction with peptides

Given that the binding of Raf-1 to Rb facilitates cell proliferation and since both proteins are known to affect the oncogenic process, we examined the feasibility of targeting Rb-Raf-1 interaction to inhibit cell proliferation and tumor growth. Towards this purpose, a peptide corresponding to amino acids 10–18 of Raf-1 was synthesized to disrupt the Rb-Raf-1 interaction (21). The peptide sequence was ISNGFGFK, and a C was added to the carboxyl terminal end to allow coupling to the carrier molecule penetratin. The Raf-1 peptide (1μM) inhibited the Rb-Raf-1 interaction without inhibiting the binding of other proteins to Rb or Raf-1 (21). The Raf-1 peptide-penetratin conjugate disrupted the binding of Rb-Raf-1 in cells, as seen in colocalization experiments as well as several other biochemical assays. Rb phosphorylation was detected at two hours after serum stimulation, when Raf-1 is found to bind to Rb, but prior to the binding of cyclin D. More surprisingly, the inhibition of Rb-Raf-1 by the Raf-1 peptide pen-conjugate could significantly inhibit Rb phosphorylation even up to 16 hours post serum stimulation (21). Since Raf-1 binding to Rb does not cause E2F1 to dissociate yet could reverse Rb mediated repression of E2F1, it was examined how Raf-1 de-represses E2F1. Chromatin Immunoprecipitation assays (ChIPs) and Immunoprecipitation western blot assays revealed that Raf-1 binding to Rb led to the dissociation of chromatin remodeling protein Brg1 from Rb (21). Although other corepressors are present, Raf-1 seems to specifically dissociate Brg1 from the promoters of E2F regulated genes. Treatment with the Raf-1 peptide-penetratin conjugate led to Brg1 recruitment on proliferative promoters. There was no change in the binding of HDAC1 and HP1. Disruption of the Rb-Raf-1 interaction with the Raf-1 peptide-penetratin conjugate could inhibit 50% of cells from entering S-phase and efficiently inhibited angiogenic tubule formation in matrigel assays as well as adhesion, migration and invasion of human aortic endothelial cells (HAECs) (21). An anti-angiogenic and anti-proliferative agent can be expected to inhibit tumor growth since these are hallmarks of cancer. A549 human xenograft tumor growth was inhibited approximately 80% by intratumoral administration of the Raf-1 peptide-penetratin conjugate (21). These results clearly demonstrated that disruption of Rb-Raf-1 interaction could efficiently inhibit tumor growth and angiogenesis *in vivo*.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Searching for novel disruptors of Rb-Raf-1

Although peptides are useful for targeting specific sequences of proteins to disrupt their interactions or enzymatic activity, they are of limited use as drugs. This is because they are degraded very quickly and delivery into cells is problematic. At the same time, information generated from studies on peptides can be fruitfully used to generate peptidomimetic drugs or other small molecules to target the interaction. It can be assumed that small molecules that are capable of inhibiting the Rb-Raf-1 interaction have therapeutic potential for controlling proliferative disorders such as cancer. An ELISA was used to identify compounds that could inhibit the binding of GST-Rb to GST-Raf-1. Screening of the NCI diversity library of 1,981 compounds identified two compounds, NSC-35400 and NSC-35950, which inhibited Rb-Raf-1 interaction 100% and 95% respectively at 20μM concentration. NSC-35400 and NSC-35950 each contained a benzyl-isothiourea derivative and a phenyl-based counter ion. To establish whether the benzyl-isothiourea derivative is the active component, we synthesized RRD-251, which contains chloride as the counter ion (31). ELISA analysis showed that NSC-35400 disrupted the Rb-Raf-1 interaction with an IC_{50} of 81 ± 4nM, NSC-35950 with an IC_{50} of 283 ± 46nM and RRD-251 with an IC_{50} of 77 ± 3.6 nM, suggesting that the benzylisothiouronium pharmacophore disrupts the Rb-Raf-1 interaction. ELISAs showed that these disruptors were highly selective for Rb/Raf-1 interaction over Rb/E2F1, Rb/HDAC1, Rb/prohibitin and Raf-1/MEK associations at a concentration of 20μM. Examination of selectivity and specificity in whole cells revealed that treatment with RRD-251 could inhibit Rb-Raf-1 without disrupting the binding Rb to E2F1, cyclin E, or B-Raf, experiments showed that RRD-251 could inhibit the Rb-Raf-1 interaction in whole cells with an IC_{50} of 450 nM. Biochemical studies on RRD-251 indicate that this molecule is selective and specific for targeting Rb-Raf-1 *in vitro* and in intact cells (31).

Anti-cancer properties of RRD-251

RRD-251 was found to inhibit cell proliferation in a wide panel of cancer cell lines of varying origin. Cell lines harboring a variety of mutations such as EGFR, Ras, p53, p16, and PTEN all responded to treatment with RRD-251 (31). In addition, melanoma and pancreatic cancer cell lines were very sensitive to treatment with RRD-251. Inhibition of S-phase entry by RRD-251 was dependent on a functional Rb protein; cell lines with a conditional deletion of Rb or cell lines with a naturally occurring mutation in Rb did not respond to treatment. Treatment with RRD-251 prevented nicotine induced cell cycle progression. Disruption of Rb-Raf-1 by RRD-251 also prevented anchorage independent growth as seen in soft agar colony formation assays. Treatment with RRD-251 was found to inhibit E2F mediated transcription as well as inhibit E2F regulated proliferative genes, *cdc6* and *thymidylate synthase (TS)*.

Since Raf-1 and Rb have both been shown to play an important role in angiogenesis, we reasoned that disruption of the Rb-Raf-1 interaction could prevent angiogenesis. Treatment with RRD-251 decreased the VEGF levels in the lung cancer cell line A549. RRD-251 prevented angiogenic tubule formation and rat aortic ring sprouting in matrigel-- two standard methods to measure *in vitro* angiogenesis. RRD-251 also significantly inhibited angiogenesis *in vivo*. Treatment with RRD-251 blocked neo-angiogenesis in nude mouse models (Table 1) (31).

Treatment of nude mice bearing human tumor cell xenografts showed that RRD-251 could inhibit tumor growth *in vivo*. RRD-251 inhibited A549 human NSCLC tumor growth by i.p. administration at 50 MPK or oral administration at 150 MPK (Table 1). RRD-251 also significantly inhibited the H1650 NSCLC tumor growth by i.p administration at 50 MPK. The melanoma cell line SK-MEL-28 was implanted with matrigel and RRD-251 could prevent SK-EL-28 tumor growth by i.p. injection of 50 MPK. Tumors containing a conditional deletion of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Rb did not respond to treatment with RRD-251, proving the selectivity of this molecule *in vivo* (Table 1). Further examination of A549 tumors from mice treated with RRD-251 showed that the Rb-Raf-1 interaction was disrupted in the tumors. These tumors also revealed an inhibition of proliferative and angiogenic markers.

Looking towards the future: targeted therapy in patients

The Ras/Raf/MAPK signaling cascade is often activated in cancers and has been sought after as a target for cancer therapies for many years. It has been established that Rb is a target for the Raf-1 kinase and the Rb-Raf-1 interaction is elevated in cancer. Disruption of the interaction has shown desirable outcomes for preventing hallmarks of cancer. Identifying small molecules capable of disrupting protein-protein interactions has been difficult, but the recent successes seem to be promising in preclinical experiments and some are currently undergoing phase I and II clinical investigation (Bcl-X_L Bak disruptors). Identification of a new class of nhibitors targeting the Rb-Raf-1 protein-protein interaction display attractive anti-cancer properties and further experiments may lead to the transition from preclinical to phase I trials in the near future (Figure 1). Since the mechanisms behind nicotine proliferative and angiogenic signaling identified the Rb-Raf-1 interaction to play a role, it only seems reasonable that lung cancer patients with a history of smoking could be ideal for personalized medicine targeting Rb-Raf-1. Because of our preclinical results with RRD-251 preventing tumor growth but not completely regressing tumors, we can assume that future clinical trials would be therapy consisting of two drugs, one aimed at targeting Rb-Raf-and the other with a DNA damaging agent or apoptosis inducer. Targeting the Rb-Raf-1 protein-protein interaction is likely a better alternative to targeting Raf-1 kinase which has roles in normal cell physiology in addition to cancer signaling pathways. Much like growth factor receptor signaling is elevated in tumors, it can be imagined that the Raf-1-Rb pathway and cell cycle progression is also constitutively driven in cancer.

Acknowledgments

Our thanks to the laboratories of Drs. Nicholas Lawrence and Said Sebti for their help with this project. This study was supported by the grants CA63136 and CA118120 from the National Cancer Institute. R.D. is the recepient of a pre-doctoral award from the American Heart Association, Florida Chapter.

References

1. Knudsen ES, Knudsen KE. Exp Biol Med (Maywood) 2006;231:1271–1281. [PubMed: 16816134]
2. Wang S, Ghosh RN, Chellappan SP. Mol Cell Biol 1998;18:7487–7498. [PubMed: 9819434]
3. Dasgupta P, Rastogi S, Pillai S, Ordonez-Ercan D, Morris M, Haura E, Chellappan S. J Clin Invest 2006;116:2208–2217. [PubMed: 16862215]
4. Bartkova J, Lukas J, Strauss M, Bartek J. Oncogene 1995;10:775–778. [PubMed: 7862456]
5. Bellacosa A, Almadori G, Cavallo S, Cadoni G, Galli J, Ferrandina G, Scambia G, Neri G. Clin Cancer Res 1996;2:175–180. [PubMed: 9816104]
6. Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C, Barnes D, Peters G. Cancer Res 1994;54:1812–1817. [PubMed: 8137296]
7. Sheyn I, Noffsinger AE, Heffelfinger S, Davis B, Miller MA, Fenoglio-Preiser CM. Hum Pathol 1997;28:270–276. [PubMed: 9042789]
8. Yatabe Y, Nakamura S, Seto M, Kuroda H, Kagami Y, Suzuki R, Ogura M, Kojima M, Koshikawa T, Ueda R, Suchi T. Am J Surg Pathol 1996;20:1110–1122. [PubMed: 8764748]
9. Geradts J, Kratzke RA, Niehans GA, Lincoln CE. Cancer Res 1995;55:6006–6011. [PubMed: 8521382]
10. Kratzke RA, Greatens TM, Rubins JB, Maddaus MA, Niewoehner DE, Niehans GA, Geradts J. Cancer Res 1996;56:3415–3420. [PubMed: 8758904]
11. Otterson GA, Kratzke RA, Coxon A, Kim YW, Kaye FJ. Oncogene 1994;9:3375–3378. [PubMed: 7936665]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

12. Sakaguchi M, Fujii Y, Hirabayashi H, Yoon HE, Komoto Y, Oue T, Kusafuka T, Okada A, Matsuda H. Int J Cancer 1996;65:442–445. [PubMed: 8621224]
13. Shapiro GI, Edwards CD, Kobzik L, Godleski J, Richards W, Sugarbaker DJ, Rollins BJ. Cancer Res 1995;55:505–509. [PubMed: 7834618]
14. Dyson N, Howley PM, Munger K, Harlow E. Science 1989;243:934–937. [PubMed: 2537532]
15. Munger K, Werness BA, Dyson N, Phelps WC, Harlow E, Howley PM. Embo J 1989;8:4099–4105. [PubMed: 2556261]
16. Kitagawa M, Taya Y. Tanpakushitsu Kakusan Koso 1997;42:1594–1602. [PubMed: 9279086]
17. Taya Y. Trends Biochem Sci 1997;22:14–17. [PubMed: 9020586]
18. D'Abaco GM, Hooper S, Paterson H, Marshall CJ. J Cell Sci 2002;115:4607–4616. [PubMed: 12415005]
19. Garnovskaya MN, Mukhin YV, Vlasova TM, Grewal JS, Ullian ME, Tholanikunnel BG, Raymond JR. J Biol Chem 2004;279:24899–24905. [PubMed: 15069084]
20. Downward J. Curr Biol 1997;7:R258–R260. [PubMed: 9162506]
21. Dasgupta P, Sun J, Wang S, Fusaro G, Betts V, Padmanabhan J, Sebti SM, Chellappan SP. Mol Cell Biol 2004;24:9527–9541. [PubMed: 15485920]
22. Hood JD, Cheresh DA. Cold Spring Harb Symp Quant Biol 2002;67:285–291. [PubMed: 12858551]
23. Kinkade R, Dasgupta P, Chellappan S. Curr Can Ther Rev 2006;2:305–314.
24. Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, Simantov R, Kelley S. Nat Rev Drug Discov 2006;5:835–844. [PubMed: 17016424]
25. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA. Cancer Res 2004;64:7099–7109. [PubMed: 15466206]
26. Heimbrook DC, Oliff A. Curr Opin Cell Biol 1998;10:284–288. [PubMed: 9561854]
27. de Carcer G, de Castro IP, Malumbres M. Curr Med Chem 2007;14:969–985. [PubMed: 17439397]
28. Malumbres M, Pevarello P, Barbacid M, Bischoff JR. Trends Pharmacol Sci 2008;29:16–21. [PubMed: 18054800]
29. Berthet C, Aleem E, Coppola V, Tessarollo L, Kaldis P. Curr Biol 2003;13:1775–1785. [PubMed: 14561402]
30. Malumbres M, Sotillo R, Santamaria D, Galan J, Cerezo A, Ortega S, Dubus P, Barbacid M. Cell 2004;118:493–504. [PubMed: 15315761]
31. Kinkade R, Dasgupta P, Carie AE, Pernazza D, Carless M, Pillai S, Lawrence N, Sebti SM, Chellappan S. Cancer Research 2008;68:3810–3818. [PubMed: 18483265]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

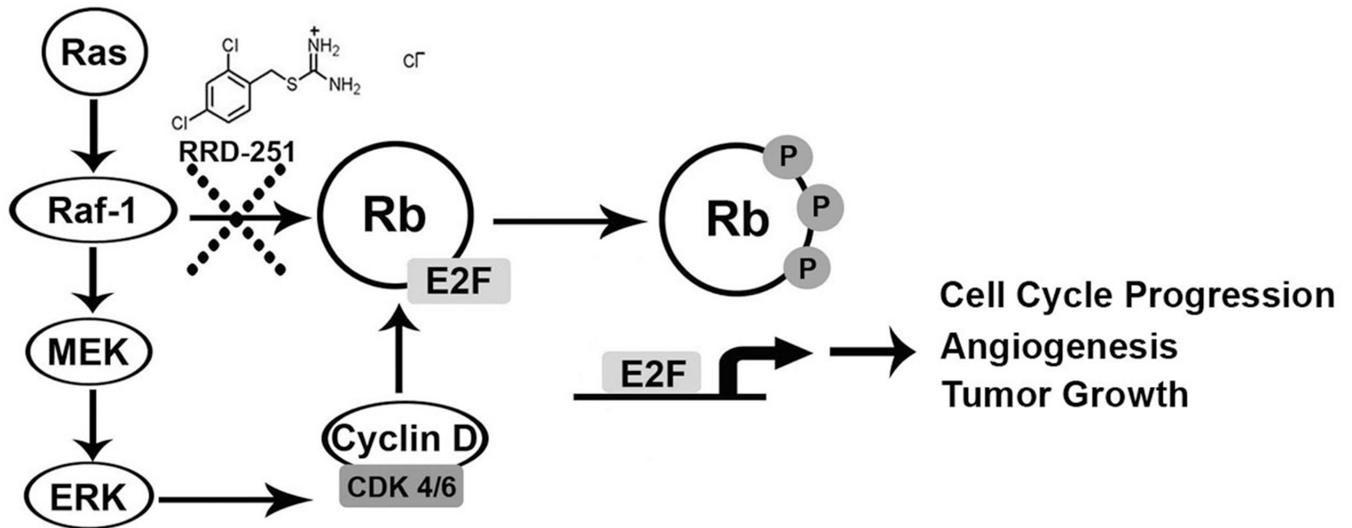

Figure 1. Schematic depicting RRD-251 inhibition of Raf-1-Rb signaling pathway

Raf activation signals through MEK/ERK pathway and Rb pathway. Activation of ERK leads to induction of CDK4/cyclin D activity and Rb phosphorylation. Raf-1 also binds and phosphorylates Rb leading to dissociation of E2F, and expression of genes involved in cell cycle progression, angiogenesis and tumor growth. Treatment with RRD-251 disrupts RB-Raf-1 interaction and prevents Rb phosphorylation and inhibits E2F-mediated transcription of S-phase genes.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Results of RRD-251 treatment *in vivo*

Experiment	Dosage/Route	Results	p value
***in vivo* Matrigel plug angiogenesis**	50 MPK i.p.	50MPK: 6-fold reduction in relative angiogenesis	0.0004
A549 Xenograft	50 MPK i.p 150 MPK-Oral	Vehicle:1040±128mm^3 50 MPK: 145±20 mm^3 150 MPK: 148±38 mm^3	50 MPK:0.0008 150 MPK:0.0004
H1650 Xenograft	50 MPK i.p.	Vehicle:2185±326mm^3 50 MPK:557±76 mm^3	0.003
SK-MEL-28/Matrigel Xenograft	50 MPK i.p.	Vehicle:861±106mm^3 50 MPK:341±42 mm^3	0.0003