Identification of the potential inhibitors of DNA polymerase kappa

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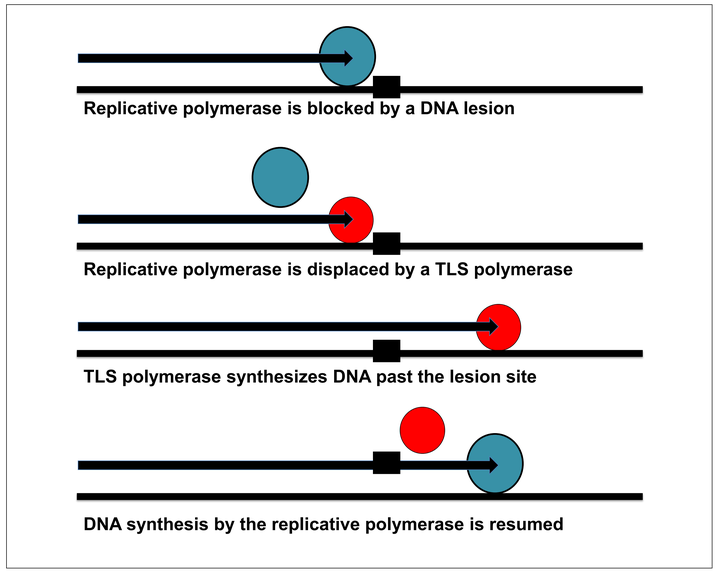
Introduction

The therapeutic effect of many anticancer agents is due to induction of replication-blocking DNA lesions, such as covalently cross-linking complementary DNA strands. Human DNA polymerase kappa (pol κ) is a translesion synthesis (TLS) polymerase that can catalyze direct replication past minor groove lesions, such as the one induced by mitomycin C. Although TLS is an essential process for the viability of cells, pol κ may decrease the efficacy of these anticancer agents. Pol κ has been found to be upregulated in ~70% of gliomas and its upregulation associated with poorer prognosis in glioma patients. Gliomas are the most common primary brain tumor in humans and are currently incurable. These tumors are resistant to standard chemotherapeutic agents and median survival time is one year after diagnosis. Therefore, small molecule inhibitors of pol κ, in combination with chemotherapy, may be useful in the treatment of cancer with upregulated pol κ expression. The specific aim of this project was to identify inhibitors of DNA polymerase kappa.



Materials & Methods

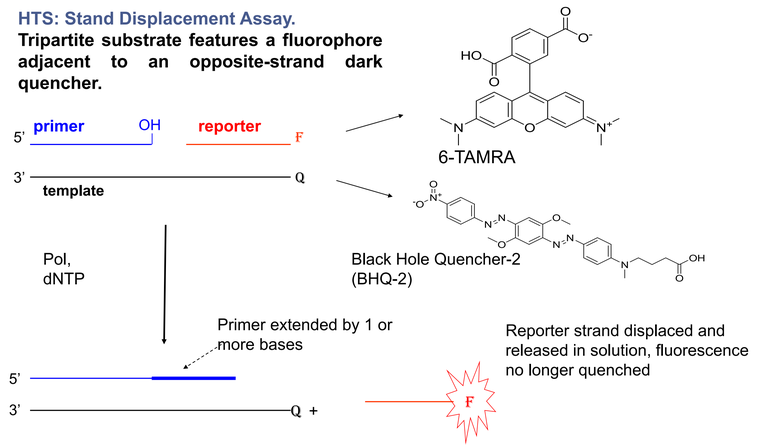
High throughput screens: The National Chemical Genomics Center conducted an initial screen of 400,000 small molecules against polymerases (κ, β, η, ι, Klenow fragment of *E. Coli* pol I, HIV-1 reverse transcriptase), helicases, topoisomerases, exonucleases, glycosylases, and abasic site endonucleases. 10,000 of these small molecules were subsequently rescreened against all the previously mentioned enzymes. Of this group, 335 small molecules were specifically identified to interact with polymerase κ.

A direct, gel-based primer extension assay: The assay was used to test for inhibitory effects of these 335 bioactive compounds. In this assay, pol κ was preincubated with individual compounds and added to ^{32}P-labeled DNA substrate to initiate primer extensions. The reactions were incubated for 30 minutes at room temperature and terminated by the addition of a DNA-denaturing solution. The products were then separated through a 15% acrylamide gel and visualized with a PhosphorImager screen.

DNA intercalation assay: Compounds that significantly inhibited the polymerization activity of pol κ were further tested for the ability to intercalate into DNA by incubation with double-stranded DNA for 15 minutes at room temperature, followed by separation through a 1% agarose gel. DNA was stained with ethidium bromide and visualized by Alpha Innotech ChemiImager.

HTS: Stand Displacement Assay.

Tripartite substrate features a fluorophore adjacent to an opposite-strand dark quencher.

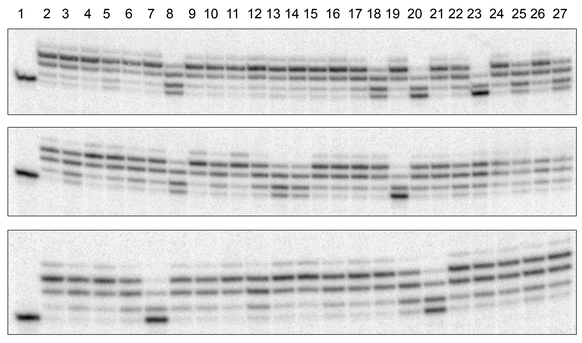


Results

Direct, gel-based primer extension assay

Lane 1: negative control
Lane 2: positive control (pol κ)
Lanes 3-27: pol κ & small molecule inhibitor

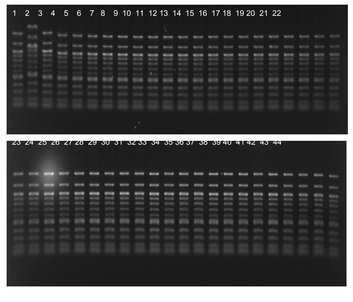
A total of 42 small molecule inhibitors of pol κ, with the estimated potencies being the low micromolar range or less.



DNA intercalation assay

Lane 1: negative control
Lane 2: positive control (pol κ)
Lanes 3-44: pol κ & small molecule inhibitor

All 42 small molecules were confirmed not to exhibit their inhibitory effects through DNA intercalation.



1	Negative control
2	Positive control (EtBr)
3	MLS002245154-01
4	MLS000676656-01
5	MLS000689854-01
6	MLS002695220-01
7	MLS001179607-01
8	MLS000737267-01
9	MLS000705142-01
10	MLS000572643-01
11	MLS000685678-01
12	MLS000554788-01
13	MLS002694133-01
14	MLS003115632-01
15	MLS001199626-01
16	MLS001242877-01
17	MLS000682713-01
18	MLS001210337-01
19	MLS001197150-01
20	MLS000562001-01
21	MLS000720395-01
22	MLS000567569-01
23	MLS001221527-01
24	MLS002182612-01
25	MLS000778776-01
26	MLS001303550-01
27	MLS000573244-01

28	MLS000673045-01
29	MLS000689657-01
30	MLS001196670-01
31	MLS000684562-01
32	NCGC00161808-01
33	NCGC00098127-01
34	NCGC00100307-01
35	NCGC00098944-01
36	NCGC00181389-01
37	NCGC00115716-01
38	NCGC00105230-01
39	NCGC00105896-01
40	NCGC00105373-01
41	NCGC00182409-01
42	NCGC00132363-01
43	NCGC00098561-01
44	NCGC00100309-01

Conclusions

Many traditional anticancer agents exhibit their effect through induction of DNA blocking lesions. However, in certain cancers, such as gliomas, human DNA polymerase κ may be rescuing the cell from deleterious effects of the anticancer agent, promoting resistance and survival. Thus, small molecule inhibitors may render tumor cells more susceptible to anticancer agents or radiation therapy.

From an initial screen of 400,000 small molecules, 335 were triaged against a rigorous screening process, including a direct, gel-based primer extension assay. 42 small molecules were identified to show specific inhibition of polymerase κ. A DNA intercalation assay determined that their inhibitory effects were not due to incorporation into the DNA. Further investigation and characterization of small molecule inhibitors will allow for a more efficient therapeutic approach in patients, who have upregulated pol κ receiving chemotherapy.

Future Directions

- Conduct primer extension assays with other Y family polymerases to ensure specificity to pol κ
- Determine if the inhibitors have common structures amenable to medicinal chemistry
- Synthetic lethality with radiation or chemotherapy in cell culture models

Summary

- Initial 400,000 small molecules were screened against various polymerases, helicases, topoisomerases, exonucleases, glycosylases, and abasic site endonucleases
- 335 small molecules specific for polymerase κ were identified
- A direct, gel-based primer extension assay identified 42 small molecule inhibitors of polymerase κ in the low micromolar range or less
- All were confirmed not to exert their inhibitory effects through a DNA intercalation assay

Acknowledgments

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www.ohsu.edu/radmedicine

References

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