CarcSeq detection of a dose- and time-dependent induction of a cancer driver mutation in the mammary DNA of lorcaserin-treated rats

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Abstract

Lorcaserin, a drug for weight management, is a selective agonist of the serotonin (5-hydroxytryptamine) 2C receptor. Although lorcaserin is a non-genotoxic rat carcinogen, FDA approval was granted in part based on dose extrapolation considerations. A post-marketing study, CAMELLIA-TIMI, designed to detect potential cardiovascular effects of lorcaserin therapy detected excess cancer risk in the lorcaserin treatment arm. Consequently, a study of lorcaserin-treated rats was conducted to elucidate the mechanism of lorcaserin-induced carcinogenesis and facilitate detection of other carcinogens operating through the same mechanism in the future. Another study goal was to characterize CarcSeq utility in detecting the neoplasia-related effects of a non-genotoxic carcinogen. CarcSeq is an error-corrected next-generation sequencing method for quantitation of panels of hotspot cancer driver mutations (CDMs) and can detect mutations with mutant fractions (MFs) $\geq 10^{-4}$. Female Sprague Dawley rats were treated by gavage daily with 0, 30, or 100 mg/kg lorcaserin, replicating the tumor bioassay doses but with shorter duration treatments of 12 or 24 weeks. Lorcaserin and N-nitroso-lorcaserin were quantified in dosing solutions, terminal plasma and terminal liver samples using ultra high-performance liquid chromatography-electrospray tandem mass spectrometry. N-nitroso-lorcaserin was not detected. Mammary DNAs (n = 6/group) were used to synthesize PCR products from genes containing known hotspot CDMs (*Apc*, *Braf*, *Egfr*, *Hras*, *Kras*, *Nfe2l2*, *Pik3ca*, *Setbp1*, *Stk11*, and *Tp53*) and variant MFs were quantified by CarcSeq. Considering MFs in all targets, no significant effects of lorcaserin treatment were observed. However, significant induction of *Pik3ca* H1047R mutation was observed after 12 and 24 weeks of treatment (ANOVA, $P<0.05$), with greater numbers of mutants and mutants with higher MFs observed in 24-week samples compared to 12-week samples. Given that *Pik3ca* H1047R mutation can be detected in normal tissues, is the most prevalent mutation in human breast cancer, and occurs in several other cancers, these results suggest lorcaserin-induced carcinogenesis involves promoting the outgrowth of spontaneously-occurring *Pik3ca* H1047R mutant clones. The underlying mechanism(s) of promotion are under investigation. This study provides the first demonstration that CarcSeq can identify the carcinogenic impact of a non-genotoxic carcinogen, doing so within a shorter timeframe than is needed to measure a tumor response.

Introduction

Better tools for predicting carcinogenicity are needed for nonclinical programs supporting development of chronically administered drugs. This study was conducted to elucidate the mechanism of lorcaserin induced rat mammary carcinogenesis and investigate the utility of CarcSeq (an error-corrected NGS method that can quantify ≥3 mutant DNA molecules in a background of 30,000 wild-type). By analyzing DNA samples from short-term, repeat-dose rodent studies and identifying putative clonal expansions of rodent homologues of human hotspot cancer driver mutations (CDMs), CarcSeq may provide an early surrogate of neoplasia development. A two-year bioassay found significant increases in mammary fibroadenoma induced by 10, 30, and 100 mg/kg lorcaserin, and mammary adenocarcinoma induced by 100 mg/kg lorcaserin in female rats (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/022529Orig1s000PharmR.pdf). Thus, CDMs in rat mammary DNA were quantified following 12 or 24 weeks of treatment with tumorigenic doses of lorcaserin (30 and 100 mg/kg).

Materials and Methods

8-week-old female Sprague Dawley rats were treated daily by gavage with 0, 30, or 100 mg/kg of lorcaserin hydrochloride (provided by Eisai Co. Ltd.) for 12 or 24 weeks. To address the concern that a genotoxic impurity might be responsible for observed lorcaserin induced carcinogenicity, lorcaserin and *N*-nitroso-lorcaserin were measured in the test article at the beginning and end of the study, in a subset of the dosing solutions and in terminal plasma and liver samples using ultra high-performance liquid chromatography-electrospray tandem mass spectrometry. At the end of the treatment period, rats were euthanized and collected mammary tissues flash frozen in liquid nitrogen. DNA was isolated from ¼ of the mammary tissue of each rat, using 6 rats for each dose and timepoint. CarcSeq was performed as depicted in Figure 1. Two independent CarcSeq analyses were performed on the DNA from rats treated for 24 weeks, to analyze CarcSeq reproducibility. The output of CarcSeq is mutant fraction (MF), defined as #mutant bases/#reference bases at any position. Statistical analyses of MFs across groups used Kruskal-Wallis tests (Dunn's multiple comparisons).

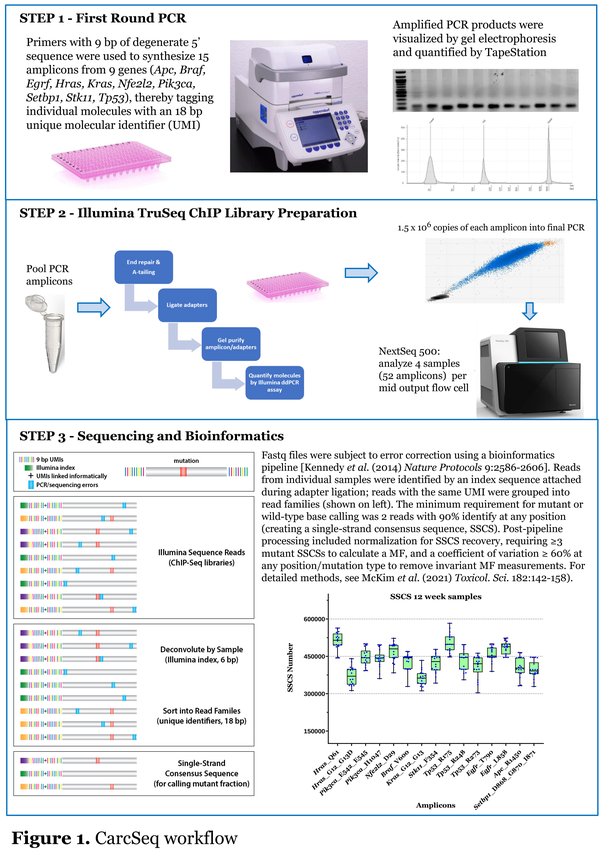

Figure 1. CarcSeq workflow

Results and Discussion

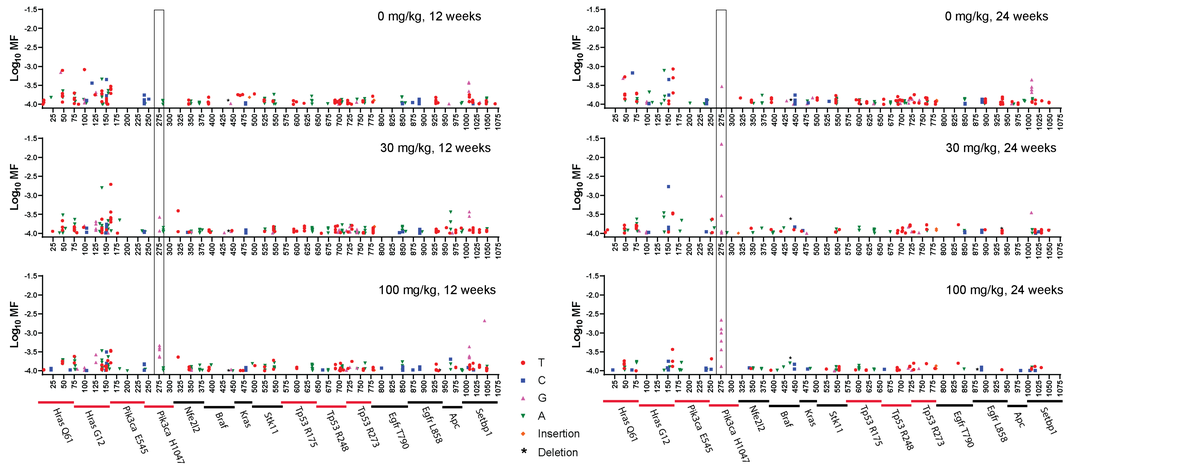

Figure 2. CarSeq detected >1,100 somatic mutants with MFs $\geq 10^{-4}$. Aside from *Pik3ca*, MFs were not significantly different across doses and timepoints. Rodent mutations equivalent to the known human breast cancer hotspot mutation, *PIK3CA* H1047R are identified in rectangles.

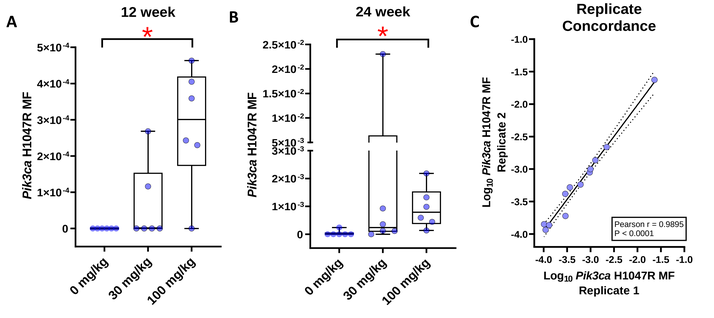

Figure 3. Time- and dose-dependent increase in *Pik3ca* H1047R MF were observed after 12 (A) and 24 weeks (B) of lorcaserin treatment. Magnitudes of the CarcSeq MF measurements are consistent with lorcaserin-induced clonal expansion of *Pik3ca* H1047R mutation. DNA samples from rats treated for 24 weeks were analyzed in two independent CarcSeq experiments. The significant correlation observed for replicate measurements (C) demonstrates reproducibility. When a *Pik3ca* H1047R MF was not detected (meaning $<10^{-4}$) in a rat, it was not detected in either replicate.

Conclusion

- Chemical and nuclear magnetic resonance analyses confirmed: 1) the identity of the test article, 2) the lorcaserin concentrations of dosing solutions, 3) lorcaserin levels in terminal plasma and liver, and 4) that N-nitroso-lorcaserin was not present in any of the samples analyzed. These results confirm that lorcaserin is a non-genotoxic carcinogen and the rat carcinogenicity bioassay conditions were replicated appropriately.
- The CarcSeq analysis demonstrated that lorcaserin induced dose-dependent increases in *Pik3ca* H1047R mutants in rat mammary DNA, with MFs increasing with duration of treatment. This observation has clear human relevance because the *PIK3CA* H1047R mutations is the single most prevalent point mutation observed in human breast cancer.
- Given that lorcaserin is a non-genotoxic carcinogen and *Pik3ca* H1047R mutation occurs in normal rat and human mammary tissues, these findings suggest that lorcaserin induces mammary carcinogenesis by promoting the proliferation of spontaneously-occurring *Pik3ca* H1047R mutant clones.
- This study provides proof-of-principle that CarcSeq can be used to detect the effects of a non-genotoxic carcinogen.