DIAGNOSTIC DETECTION OF DNA REPAIR PATHWAY ABERRATIONS IN CANCER

PhD thesis

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Budapest
2021

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Introduction

Diverse DNA repair mechanisms are essential for the maintenance of genomic integrity in normal cells. DNA repair deficiency can lead to accumulation of cancer-initiating mutations, and tumor DNA repair pathway aberrations also contribute to the accumulation of mutations that enhance survival and fitness of cancer cells. Consequently, due to their major contribution to genomic instability, DNA repair pathway aberrations are hallmark biological determinants of the cancerous process.

Importantly, the very same DNA repair pathway aberration that may have initiated and/or promoted cancer development may also represent a significant therapeutic vulnerability. Because DNA repair pathway aberrations are common in tumor cells but are largely absent in normal cells, therapeutic agents that target DNA repair deficient cells may have a clinically exploitable therapeutic window. A successful example stemming from this principle is the development of PARP inhibitors. Cells with homologous recombination (HR) deficiency due to alterations in HR genes such as BRCA1 or BRCA2 have ~1000-fold higher sensitivity to PARP inhibitor treatment compared to cells with intact HR. This synthetic lethal relationship has been successfully exploited for the treatment of BRCA1/2 mutant cancers and four PARP inhibitors have been approved by the FDA for the treatment of ovarian, breast and prostate cancer.

A major obstacle in the optimal exploitation of therapeutic agents that are synthetic lethal with specific DNA repair pathway aberrations is the lack of reliable diagnostic methods to identify and quantify the various DNA repair pathway deficiencies in tumor biopsies.

Our work was aimed at providing solutions for this diagnostic problem. We developed methods for the accurate detection of homologous recombination deficiency in solid tumors, and thus improve the efficacy of PARP inhibitor-based therapy. We were also exploring the diagnostic detection of other DNA repair pathway aberrations, such as nucleotide excision repair, and how those could be targeted in the therapeutic setting.

Specific Aims:

1) Developing a clinically applicable diagnostic method to detect homologous recombination deficiency in tumor biopsies.

2) Diagnostic detection of homologous recombination deficiency in solid tumor types that are usually not associated with germline BRCA1 and BRCA2 mutations.

3) Diagnostic detection of homologous recombination deficiency in the metastatic setting.

4) Developing a clinically applicable diagnostic method to detect nucleotide excision repair deficiency in tumor biopsies.

Methods:

The core principle of our work is based on the observation that DNA repair pathway aberrations in cancer will lead to the introduction of mutations into the genome of the tumor cells. These mutations can be detected by next generation sequencing of the entire exome (whole exome sequencing, WES) or the entire genome (whole genome sequencing, WGS) and the mutational profiles detected in a given genome are characteristic of the aberrant DNA repair pathway or genotoxic agent that has led to the accumulation of mutations. For example, loss of function of BRCA1 will lead to a characteristic mutational profile of single nucleotide substitutions, short deletions flanked by microhomologies and large-scale rearrangements (tandem duplications of several kb long DNA regions). (Reviewed in **publication 4**)

In most of our work we used either whole exome or whole genome sequencing data derived from the DNA of tumor biopsies.
We analyzed data from a wide variety of sources. In **publication 1** we analyzed SNP array data from a neoadjuvant platinum treated triple negative breast cancer data set. In **publication 2** we analyzed previously published SNP array and whole genome and whole exome sequencing based breast cancer data sets available from TCGA. In **publication 3** we analyzed previously published whole exome sequencing data from patient matched primary breast cancer/brain

metastasis pairs. We also performed HRD score analysis on our own cohort of patient matched primary breast cancer/brain metastasis pairs. In **publication 4** we analyzed whole exome and whole genome sequencing data from several previously published prostate cancer cohorts. In **publication 5** we analyzed whole exome and whole genome sequencing data from several previously published bladder cancer cohorts.

The raw sequencing data were routinely processed as follows: Germline mutations were called with HaplotypeCaller; somatic point-mutations and indel were called using Mutect2 (GATK 3.8). The high fidelity of the reported variants was ensured by the application of additional hard filters on top of the tools' default ones. Allele-specific copy-number profiles were estimated by using Sequenza. Structural Variants were called using BRASS (v6.0.0; https://github.com/cancerit/BRASS). Genomics scar scores (loss-of-heterozygosity, LOH; large scale transitions, LST; and number of telomeric allelic imbalances, ntAI were determined using the scarHRD R package as described in publication 4. We also developed several novel complex mutational signatures such as that described in **publication 5**, a complex mutational signature associated with ERCC2 mutations.

Results:

1) Developing a clinically applicable diagnostic method to detect homologous recombination deficiency in tumor biopsies.

Homologous recombination deficiency was initially described and defined in the context of germline and somatic loss of function mutations of BRCA1 and BRCA2. However, it has become evident that tumors without mutations in these key homologous recombination enzymes may also be homologous recombination deficient. In the absence of this DNA repair mechanism (HR) specific mutational patterns accumulate in the tumor DNA and we identified the first such "DNA scarring signature", telomeric allelic imbalance (see **publication 1**). This method has become the first component of an FDA approved diagnostic mutational signature, the myChoice HRD assay. This is routinely used now for the prioritization of ovarian cancer patients for PARP inhibitor therapy. Our method was initially developed for SNP arrays. However, next generation sequencing has largely replaced this hybridization-based technology and we converted and validated our method for next generation sequencing based platforms (see **publication 2**).

2) Diagnostic detection of homologous recombination deficiency in solid tumor types that are usually not associated with germline BRCA1 and BRCA2 mutations.

Homologous recombination deficiency was described in the context of the loss of function mutations of BRCA1 and BRCA2. Therefore, the efficacy of PARP inhibitors, the agents specifically targeting homologous recombination deficient tumors, was first explored in tumor types that are often associated with such mutations, namely ovarian and breast cancer. However, homologous recombination deficiency can be present even in the absence of BRCA1 or BRCA2 mutations in ovarian and breast cancer and those could be detected by the HR deficiency associated mutational signatures (see **publication 1**). Furthermore, homologous recombination deficiency can be present in cancer types that are more rarely or only occasionally associated with BRCA1/2 mutations. (In prostate cancer BRCA1/2 mutations are present with a frequency of 5% or less, in lung and bladder cancer BRCA1/2 mutations are present with a frequency of 1% or less). Therefore, we decided to determine the frequency of homologous recombination deficient cases, as detected by HR deficiency associated mutational signatures, in these tumor types. We have completed and published our first analysis (prostate cancer, see **publication 4**) and found that in addition to the ~5% cases with direct loss of function mutations in the HR genes (BRCA1/2, PALB2 etc.) an additional 5-10% cases show strong signs of HR deficiency in prostate cancer cohorts. This suggests that the potentially PARP inhibitor sensitive population of prostate cancer cases is larger and our work will have

implications for the design of the current clinical trials of PARP inhibitors in prostate cancer.
We are also preparing publications on the frequency of HR deficiency associated mutational signatures in lung and bladder cancer.

3) Diagnostic detection of homologous recombination deficiency in the metastatic setting.

We also investigated whether HR deficiency associated mutational signatures differ in primary tumors and their matched metastatis. In particular, we compared the various HR deficiency induced genomics aberrations in primary breast cancer and their patient matched brain metastasis (see **publication 3)**. We found that the various genomics measures of HR deficiency were significantly higher in the brain metastases relative to the primary tumors. In several instances this increase was so significant that the HR deficiency negative primary tumor had an HR deficiency positive brain metastasis. This has clinical consequences. While the primary tumor may be resistant to PARP inhibitor therapy, its brain metastasis may be sensitive to this treatment. Therefore, breast cancer metastases may need to be reassessed for HR deficiency for optimal therapeutic decisions.

4) Developing a clinically applicable diagnostic method to detect nucleotide excision repair deficiency in tumor biopsies.

We also explored whether specific diagnostic mutational signatures can be derived for other types of DNA repair pathway aberrations as well. We were particularly interested in nucleotide excision repair (NER) deficiency. Preclinical studies suggested that the experimental cancer therapy drug, irofulven, may possess a synthetic lethal efficacy against NER deficient cancer similar to that seen for PAPR inhibitors in the context of HR deficient cancer. However, due to the lack of reliable identification of NER deficient cancer cases, this hypothesis could not be tested in advanced clinical settings. In fact, most phase 2 clinical trials of irofulven showed only limited clinical efficacy, since patients were not prioritized based on their NER status. We recently published (see **publication 5)** that ERCC2 mutant bladder cancer cases display a specific NER deficiency associated mutational signature. In addition to the 10-15% ERCC2 mutant cases an additional 10-15% of bladder cancer cases show robust signs of the same NER deficiency mutational signatures. Finally, we showed that ERCC2 mutant/NER deficient preclinical bladder cancer models show enhanced, clinically exploitable sensitivity for irofulven. These observations form the basis of a soon to be started clinical trial of irofulven treatment in bladder cancer.

Conclusions:

We showed that specific DNA repair pathway aberrations (homologous recombination and nucleotide excision repair deficiencies) are associated with diagnostically applicable mutation signatures. We developed methods to detect such signatures from either fresh frozen or formalin fixed paraffin embedded (FFPE) material. Our methods are applicable both in whole exome and whole genome sequencing.

We showed that HR deficiency can be reliably detected in solid tumor biopsies and that the diagnostic detection of such signatures has clinical relevance. It can identify patients with PARP inhibitor sensitivity in ovarian, breast and prostate cancer.

We also showed that diagnostic signatures can be derived for another DNA repair pathway aberration, nucleotide excision repair deficiency. This signature will be essential to introduce irofulven therapy to bladder cancer patients, especially for those patients that are ineligible for platinum-based therapy.

In summary, we developed diagnostic mutational signatures that can effectively direct the application of certain targeted therapies, which derive their efficacy from a synthetic lethal relationship with specific DNA repair pathway aberrations. Thus, we established clinically

applicable combinations of companion diagnostics and DNA repair pathway aberration specific therapeutic agents.

List of PUBLICATIONS included in the thesis:

1) Birkbak NJ, Wang ZC, Kim JY, Eklund AC, Li Q, Tian R, Bowman-Colin C, Li Y, Greene-Colozzi A, Iglehart JD, Tung N, Ryan PD, Garber JE, Silver DP, **Szallasi Z (co-corresponding),** Richardson AL. Telomeric Allelic Imbalance Indicates Defective DNA Repair and Sensitivity to DNA-Damaging Agents. Cancer Discov. 2012 Apr;2(4):366-375. doi: 10.1158/2159-8290. CD-11-0206. Epub 2012 Mar 22. PMID: 22576213
Impact factor: 29.497

2) Sztupinszki Z, Diossy M, Krzystanek M, Reiniger L, Csabai I, Favero F, Birkbak NJ, Eklund AC, Syed A, Szallasi Z. Migrating the SNP array-based homologous recombination deficiency measures to next generation sequencing data of breast cancer. NPJ Breast Cancer. 2018 Jul 2;4:16. doi: 10.1038/s41523-018-0066-6. eCollection 2018. PMID: 29978035
Impact factor: 6.000

3) Diossy M, Reiniger L, Sztupinszki Z, Krzystanek M, Timms KM, Neff C, Solimeno C, Pruss D, Eklund AC, Tóth E, Kiss O, Rusz O, Cserni G, Zombori T, Székely B, Tímár J, Csabai I, **Szallasi Z (corresponding)** Breast cancer brain metastases show increased levels of genomic aberration based homologous recombination deficiency scores relative to their corresponding primary tumors. Ann Oncol. 2018 Jun 18. doi: 10.1093/annonc/mdy216. PMID: 29917049
Impact factor: 18.274

4) Sztupinszki Z, Diossy M, Krzystanek M, Borcsok J, Pomerantz MM, Tisza V, Spisak S, Rusz O, Csabai I, Freedman ML,

Szallasi Z (corresponding) Detection of molecular signatures of homologous recombination deficiency in prostate cancer with or without BRCA1/2 mutations. Clin Cancer Res. 2020 Feb 18. pii: clincanres.2135.2019. doi: 10.1158/1078-0432.CCR-19-2135. [Epub ahead of print] PMID: 32071115
Impact factor: 10.107

5) Börcsök J, Sztupinszki Z, Bekele R, Gao SP, Diossy M, Samant AS, Dillon KM, Tisza V, Spisak S, Rusz O, Csabai I, Pappot H, Frazier Z, Konieczkowski D, Liu D, Vasani N, Rodrigues JA, Solit DB, Hoffman-Censits J, Plimack E, Jonathan Rosenberg J, Lazaro JB, Taplin ME, Iyer G, Brunak S, Lozsa R, Van Allen EM, Szuts D, Mouw KW, **Szallasi Z (corresponding)** Identification of a synthetic lethal relationship between nucleotide excision repair (NER) deficiency and irofulven sensitivity in urothelial cancer. Clin Cancer Res. 2020 Nov 18:clincanres.3316.2020. doi: 10.1158/1078-0432.CCR-20-3316. Online ahead of print. PMID: 33208343
Impact factor: 10.107

Acknowledgements:

I would like to thank the contributions of the following collaborators:

Tímár József, Kulka Janina, Tőkés Anna-Mária, Reiniger Lilla Rusz Orsolya, Moldvay Judit, Dióssy Miklós, Sztupinszki Zsófia, Börcsök Judit, Prósz Aurél, Csabai Istvan, Pipek Orsolya Szüts Dávid, Zámborszky Judit, Szikriszt Bernadett, Szász Marcell, Aron Eklund, Nicolai Birkbak, Andrea Richardson, Dan Silver, Charles Swanton, Kent Mouw, Marcin Krzystanek Tóth Erika, Cserni Gábor, Tisza Viktória, Spisák Sándor, Matthew Freedman, Mark Pomerantz, Ribli Dezső, Póti Ádám