DNA Repair xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Targeting Mismatch Repair defects: A novel strategy for personalized cancer treatment

Rumena Begum, Sarah A. Martin*

Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, London EC1 M 6BQ, UK

ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 8 September 2015
Accepted 30 November 2015
Available online xxx

Keywords:
Mismatch Repair
Therapies
Synthetic lethality

ABSTRACT

The DNA Mismatch Repair (MMR) pathway is a fundamental cellular process required to repair mispaired bases introduced routinely during DNA replication. Given this critical role in the maintenance of genome stability, it is not surprising that underlying defects in the MMR pathway occur in both hereditary and sporadic cancers. Furthermore, the MMR status greatly influences the sensitivity of cells to many common chemotherapeutic agents. Therefore, novel strategies are being investigated to exploit the loss of MMR in these cancers and to identify personalized therapeutic strategies to target MMR deficient tumours. In this review, we describe recent advances in strategies to target MMR deficient tumours using a synthetic lethal approach. We discuss new ways to target mutations secondary to MMR deficiency and suggest potential new therapies to optimise treatment outcome. We highlight ongoing clinical studies focussing on novel ways of preventing and treating MMR deficient cancers.

1. Introduction

The critical role for the DNA Mismatch Repair (MMR) pathway in removing DNA replication errors is imperative to the maintenance of genomic stability [1,2]. Deficiency of the MMR pathway, characterized by loss of expression of one or more of the MMR proteins (MLH1, MSH2, MSH6, PMS2, MLH3) leads to a mutator phenotype. This is due to the 100–1000-fold increased acquisition of mutations, primarily in microsatellites [2]. Microsatellites are short repetitive sequences that are scattered across the genome. Due to the repetitive nature of the sequence, loss of the MMR pathway can lead to an increase in errors during replication leading to a high rate of microsatellite instability (MSI) in MMR-deficient tumour cells [3]. Numerous cancer-related genes involved in critical processes including DNA repair, apoptosis, signal transduction and immune responses, contain microsatellites in their DNA and are therefore prone to errors in MMR deficient cancers. It is thought that the tumourigenic phenotype in MMR deficient cancers is promoted by secondary mutations in the microsatellites of these cancer-associated genes [4].

2. MMR deficiency and tumourigenesis

The essential role of the MMR pathway in cancer development is illustrated by the fact that germline mutations in MMR genes can lead to the autosomal condition; Hereditary non polyposis colorectal cancer (HNPCC), also known as Lynch Syndrome. Lynch syndrome is characterised by an 80% increased risk of developing colorectal cancer, a 20–60% and a 12–15% increased risk of women developing endometrial and ovarian cancer, respectively [5–7]. There is also an increased risk of developing other cancers such as small bowel, glioblastoma, pancreatic, urinary tract, liver, kidney, and bile duct ([6–8]; *see Sijmons & Hofstra in this issue*). Although previously prostate cancer was not thought to be under the spectrum of Lynch syndrome, recently there is evidence to suggest that male Lynch syndrome patients, have an almost five-fold increase risk of developing prostate cancer [9]. In 2012, a database of all known cancer-causing mutations in Lynch syndrome showed that the majority of Lynch syndrome-associated mutations were in *MLH1* (42%) and *MSH2* (33%), followed by *MSH6* (18%) and *PMS2* (7.5%; [10]). In this hereditary condition, only one mutated allele is passed on with subsequent loss of the second allele somatically via mutation or methylation. However, in rare cases, both of the mutated alleles may be inherited resulting in cancer in infancy, a condition known as constitutional MMR deficiency syndrome [11]. Defects in the MMR pathway can also occur as a result of somatic mutations or epigenetic silencing, by hypermethylation of the MLH1 promoter. Overall, MMR deficiency has been identified in ~15% of all colorectal cancers [12] and ~30% of all endometrial cancers [13]. Therefore, given the significant impact of MMR deficiency on numerous tumour types, there is a critical clinical need for targeted therapeutic strategies for these cancers (*see Heinen, in this issue*).

* Corresponding author. Fax: +44 20 7882 3884.
E-mail address: sarah.martin@qmul.ac.uk (S.A. Martin).

http://dx.doi.org/10.1016/j.dnarep.2015.11.026
1568-7864/

3. Treatment of MMR deficient cancers

Traditionally, the treatment options available to cancer patients involve surgery, radiotherapy and chemotherapy. To date, there is conflicting data as to whether MMR deficiency confers resistance, sensitivity or has no effect on cellular viability in response to treatment with radiotherapy [14–20]. MMR deficient cells have been shown to be radio-sensitized following acute high dose-rate irradiation [15]. However, conversely MMR deficient cells have been shown to be more resistant to low-dose irradiation [16]. This tolerance in MMR deficient cells to low dose irradiation may be due to the accumulation of DNA lesions such as oxidative clustered DNA lesions (OCDLs) or possibly O6-methylguanine (O6MeG) or O6MeG-like lesions ([16,17]; *see Li Z et al. & Crouse, in this issue*). In addition the role for the MMR pathway in the regulation of the cell cycle and the homologous recombination pathway ([15,18,19]; *see Tlam & Lebbink, in this issue*) may influence sensitivity to ionizing radiation. Further studies are required to fully elucidate the impact of the use of radiotherapy in the treatment of MMR deficient tumours. This is particularly pertinent for the treatment of tumour types where radiotherapy is routinely standard of care such as in endometrial, glioblastoma and rectal cancers, where defects in MMR frequently occur.

It is widely known that MMR-deficient cells are inherently or can acquire resistance to many of the common chemotherapeutic drugs currently used in the clinic. If we concentrate on the tumour types predominantly displaying MMR deficiency, we can quite easily identify the significant clinical problem MMR deficiency can be on the success of current treatment options. MMR deficient cells have been reported to be resistant to a number of antimetabolites including 5-fluorouracil (5-FU) and 6-thioguanine [21–23]. 5-FU-based adjuvant therapy has been the standard of care for colorectal cancer for many years. There is an increasing body of evidence showing contradictory evidence regarding whether tumours with high MSI as a result of MMR deficiency, are more or less resistant to 5-FU treatment. A number of studies, including a prospective study of clinical trial data in colorectal cancer patients, have observed sensitivity to 5-FU treatment in MMR deficient tumours [24–26]. However, *in vitro* studies in MMR deficient cells with a range of mutations including MLH1, MSH2 and MSH6, had an approximately 18-fold increase in resistance to 5-FU compared to MMR proficient cells [27]. A more recent study demonstrated in a panel of 77 colorectal cell lines that there was a strong correlation between MMR deficiency and resistance to 5-FU [28]. Clearly indicating that 5-FU based treatment regimes may not be the optimal treatment option for all of the colorectal cancer patients with loss of MMR. A number of studies have however suggested that MMR-deficient cells are more susceptible to another of the main colorectal cancer chemotherapies, irinotecan, although this may be due to to secondary mutations in DSB repair genes rather than MMR deficiency alone and therefore may only be appropriate for a small proportion of the MMR deficient colorectal cancer patients [29,30].

MMR deficiency has also been shown to potentially confer resistance to a number of platinum-based agents including cisplatin and carboplatin, widely used for the treatment of tumour types such as endometrial and ovarian cancer ([31,32]; (*see Li Z et al. & Heinen, in this issue*). Studies suggest this is an acquired resistance [33,34] with one study comparing MSI status and MMR protein expression before and after cisplatin treatment. This study showed that 73.3% of patient tumours lost MLH1 protein expression after treatment [33]. Further studies reported that patients with significantly lower MSH2 levels showed resistance to cisplatin-based chemotherapy [35]. Conversely however, Samimi et al. found no association between MSH2 and MLH1 expression with response to cisplatin, however they did observe reduced expression of both MLH1 and MSH2 after platinum-based chemotherapy [36]. In addition, studies in mouse embryonic stem cells with a targeted disruption of the *Msh2* gene did not show increased resistance to cisplatin treatment [37]. The standard of care for newly diagnosed patients with glioblastoma involves treatment with the methylating agent, temozolomide. The majority of patients become resistant to temozolomide and accumulating data has identified that up to 40% of patients recur after initial therapy due to a mutation in MSH6 [38–40]. MMR deficient cells have also been shown to be resistant to other methylating agents, including *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) and procarbazine [41,42]. The molecular mechanisms by which MMR deficiency can lead to drug resistance will be discussed in detail, by Hsieh et al. in this issue. Given the significant resistance observed in MMR deficient tumour cells to the majority of standard chemotherapies, there is a critical clinical need to identify novel therapeutic agents that will specifically target MMR deficient tumour cells.

4. Synthetic lethal targeting of MMR deficient tumours

Recent advances in high-throughput genomic, transcriptomic, and proteomic technologies have greatly increased our understanding of the molecular biology of cancer cells. The concept of "Personalized medicine" has evolved by integrating this molecular and cellular information to improve drug efficacy, based on the underlying molecular defects of individual patients tumour cells. Based on this approach, the concept of synthetic lethality has been exploited as a novel therapeutic strategy in cancer to target loss of tumour suppressor genes [43]. In this model, loss or silencing of one gene alone is compatible with cellular viability whilst simultaneous perturbation of two genes results in cell death. When you consider one of these genes as a mutation in a tumour suppressor gene, such as in an MMR gene, the second gene represents a novel therapeutic target for the selective killing of MMR-deficient cells. A number of studies have been carried out and have successfully identified synthetic lethal interactions with MMR-gene mutations.

Based on data in budding yeast identifying synthetic lethal interactions with loss of MMR genes and DNA polymerases [44], our initial studies identified that silencing of the DNA polymerases, *POLB* or *POLG*, is synthetically lethal with *MSH2* or *MLH1* deficiency, respectively in human cancer cells [45]. Significantly, POLB and POLG were also shown to be upregulated in tumour samples from patients with MSH2 and MLH1 deficient colorectal cancers, respectively. This synthetic lethal phenotype was due to an accumulation of nuclear oxidative DNA damage upon POLB silencing in MSH2-deficient cells, whilst an accumulation of mitochondrial oxidative DNA damage upon POLG silencing in MLH1-deficient cells reduced cell viability [45]. Recently, Mishra and Kowluru (2014) observed a similar requirement for MLH1 in the maintenance of mitochondrial genome stability, in retinal endothelial cells [46]. Therefore suggesting that targeting the mitochondria may be clinically applicable for the treatment of MLH1 deficient disease and furthermore, inducing oxidative DNA damage in MMR-deficient cells may provide a novel selective therapeutic strategy. The molecular mechanisms regarding MMR deficiency and oxidative DNA damage will be discussed in detail by Crouse in this issue.

Based on this hypothesis, we have also identified that MSH2 deficient cell lines *in vitro* and *in vivo* are synthetically lethal upon treatment with the oxidative damage-inducing agent methotrexate [47]. These findings have been translated to the clinic in a phase 2 clinical trial in the metastatic colorectal cancer population (NCT00952016). Furthermore, treatment with the cytosine-based analog cytarabine is also selective for MMR-deficient cells, through the induction of oxidative stress [48]. Using high-throughput small-interfering RNA (siRNA) screens, we identified that the accumulation of both nuclear and mitochondrial oxidative DNA damage, via silencing of the PTEN-inducible kinase, PINK1, induces syn-

Please cite this article in press as: R. Begum, S.A. Martin, Targeting Mismatch Repair defects: A novel strategy for personalized cancer treatment, DNA Repair (2015), http://dx.doi.org/10.1016/j.dnarep.2015.11.026