Understanding cellular pathways of DNA damage recognition and repair – clinical implications

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The primary molecular structure that carries genomic information - DNA - is constantly subject to alteration and damage by endogenous substrates and environmental factors. Damage can affect DNA in various ways, for example chemical alterations of single bases, single mismatched nucleotides, and more complex alterations such as breaks to single and double strands of DNA molecules, potentially resulting in aneuploidy, deletions, fusions or translocations. For the normal development of the individual cell and the organism as a whole, complex machinery is in place for maintaining the integrity of DNA and the genomic information carried by it to ensure normal development of the organism and prevent malignant transformation and death. The cellular pathways that are in place to recognise defects in DNA structure, and to initiate and carry out the appropriate response, which normally result in either reinstatement of the genomic integrity or cell death, are the subject of current and exciting molecular biological investigations. Damage can be processed by nucleotide excision, base excision, non-homologous or homology directed repair pathways, which all require complex protein/DNA and protein/protein interaction. Although the understanding of these pathways is still incomplete, a picture emerges that shows a cellular network of these pathways closely interacting with each other and other cellular functions involved in cell division, proliferation and differentiation. Proteins that form this network are encoded by more than 150 genes, which facilitate maintenance of DNA integrity. All of these share an interesting biology and here I will attempt to focus on aspects of this important field that are likely to be relevant for the care of children with malignant disease and their families.

Malignant diseases are common and serious complications for individuals with an inherited disruption in genes encoding for proteins involved in DNA damage response pathways. Examples are Ataxia telangiectasia (AT), Bloom syndrome (BS), Nijmegen breakage syndrome (NBS) and Fanconi anaemia (FA). AT is caused by mutations in the ATM gene, which functions as a kinase that is activated by ionising radiation and results in microcephaly, progressive cerebral degeneration and immunodeficiency. BS is caused by autosomal recessive inherited mutations in the BLM gene, which encodes for a helicase and results in short stature, UV sensitivity and immunodeficiency. NBS is an autosomal recessive disease arising from disruption of the NBS1 gene, which encodes for the double strand break repair protein nibrin that interacts with other essential double strand repair proteins such as MRE11 and RAD51. Clinically NBS shares the phenotypic feature of severe immunodeficiency with AT. Fanconi anaemia is an autosomal or X-linked inherited disease caused by mutations in at least 12 genes, which interact in a common cellular pathway. The Fanconi genes FANCA, FANCB, FANCC, FANCG, FANCF, FANCM and FANCL encode for proteins, which form a core complex that facilitates the ubiquitination of the FANCD2 protein. Ubiquitinated FANCD2 co-localises with BRCA1 at the site of DNA damage. Downstream in this pathway operate FANCI and FANC/BRCA2. On a cellular level there are important functional interactions between ATM, nibrin, BLM and the Fanconi proteins. ATM phosphorylates nibrin and FANCD2, while the FA core proteins form complexes with the BLM helicase. The detection of co-localisations signals of these proteins at the site of DNA damage suggests additional direct and indirect interactions. Despite the complex cellular interactions of these proteins and the overlapping clinical features of these disorders, the spectrum of malignancies in each is quite distinct. While AT patients are at
extremely high risk for development of lymphoid malignancies including Hodgkin’s disease, T- and B cell Lymphomas and T-cell ALL; NBS patients, in addition to the high risk of lymphomas, also develop brain tumors and rhabdomyosarcomas, which intriguingly appear to arise preferentially peri-anally. Bloom syndrome carries a very high risk of a broad range of malignancies including leukaemias, embryonal tumours and epithelial cancer. FA patients carry an extreme high risk of developing acute myeloid leukaemia (AML) and squamous cell carcinoma, but the spectrum of malignancies in the rare FA-D1 group with bi-allelic mutations in the BRCA2 gene includes brain tumours, mainly medulloblastoma, and Wilms’ tumour as well as T-cell ALL. These patients are also at extreme risk of very early AML, normally presenting within the first three years of life. It is important to consider these genetic conditions in cases that appear to be sporadic malignancies, since patients with NBS or FA can have a very subtle phenotype but still experience extreme toxicity when treated with chemotherapy or radiation, as these pathways play an important role in the cellular response to chemotherapy and radiation. Several studies and reports have suggested that inherited chromosomal fragility syndromes are frequently overlooked or unrecognised. The detection of BRCA2 as the Fanconi gene mutated in the rare FA-D1 group also implies that the correct diagnosis can have important implications for the parents and siblings in terms of cancer risk in the future, which might not be evident from a superficially taken family history.

Due to the overall rarity of these conditions the question as to whether malignancies arising from chromosomal instability syndromes have the same biology as sporadic childhood malignancies is difficult to answer. On the other hand, erroneous repair of DNA breaks has been implicated in the origin of chromosomal translocations that are characteristic for many childhood malignancies including leukaemia, lymphomas and solid malignancies. This implies the possibility that the same mechanisms that cause malignant transformation in genetic defects of DNA damage recognition and repair may be involved in sporadic childhood cancers. Little is known at present about the dynamics of the DNA damage recognition and repair machinery during prenatal development, when many childhood malignancies appear to be initiated. Whether this follows the same dynamics as in the postnatal period will be an interesting question to address in the future. Detailed cytogenetic investigations of myelodysplastic syndrome (MDS) and AML in FA patients, however, point to genetic events on chromosome 3q that are strongly associated with malignant transformation and progression in haematological malignancies arising from an inherited defect in the FA pathway, which does not appear to involve a common non-random chromosomal translocation characteristic for childhood leukaemia. Together with the observation that FA-derived AML in general lacks typical chromosomal translocations characteristic of childhood leukaemia, implies the possibility that the biology of malignancies arising from an inherited disruption in DNA damage recognition and repair might be different from that of sporadic childhood malignancies. Characterising the genetic changes also of other malignancies in patients with inherited defects will give further answers to these important questions.

Several studies have addressed the role of genetic variations and heterozygous carrier status in genes involved in DNA damage recognition and repair for childhood malignancies. These have included ATM, NBS1 and some of the FA genes as well as other DNA damage recognition and repair genes and concentrated mainly on leukaemia and lymphomas. Although several genetic variants have been identified that might confer an increased risk for certain malignancies, all of these studies were limited by relatively small patient numbers, which in general limits the value of genetic epidemiology for paediatric oncology. In adults, however, heterozygous carrier status of mutations in ATM, NBS1, BLM and certain FA-genes – especially FANCD1/BRCA2, - appear to confer an increased cancer risk, which includes haematological malignancies as well as epithelial cancer. Intriguingly the spectrum of malignancies can be very different as exemplified by one of the clinically most important cancer genes, BRCA2: While bi-allelic mutations in BRCA2 are
associated with medulloblastoma, Wilms' Tumour and very early AML, heterozygous carrier status of BRCA2 mutations gives raise to breast, ovarian and prostate cancer. This implies additional roles of the BRCA2 protein in early development that so far had little attention paid to it.

Many childhood malignancies as well as adult cancers share features of chromosomal instability and chemo-sensitivity with cells carrying a constitutional disruption in a DNA damage recognition and repair function, such as FA or NBS. This implies the possibility that in sporadic malignancies these pathways might be somatically disrupted. Somatic alteration and disruption of the ATM gene has been demonstrated in a number of sporadic lymphoid malignancies, mainly in adults, but also in children and implies a role of acquired ATM dysfunction in a proportion of sporadic lymphoid malignancies. Methylation induced silencing of the FANCF gene resulting in a acquired dysfunction of the FA pathway has been suggested to play an important role in some adult type epithelial cancers and to confer an important mechanism of platinum sensitivity. However, other studies have not detected methylation of FANCF and other FA genes in ovarian tumours and childhood leukaemia. There are, however, numerous other mechanisms that could result in somatic disruption of DNA damage response pathways and confer chromosomal instability and chemo sensitivity. These include other forms of modulation of gene expression or posttranslational modifications. Investigations addressing somatic disruption and acquired dysfunction in DNA damage recognition and repair pathways in malignant cells could prove very important in the future. Targeting the inherited DNA repair defect in BRCA2-associated breast cancer appears to be an important novel therapeutic strategy that can be pharmacologically exploited. Inhibition of the base excision repair protein Poly(ADP-ribose)polymerase (PARP) in BRCA2 deficient cells with experimental compounds has been shown to result in profound chemo-sensitisation and is currently the subject of pilot clinical investigations. These strategies might not only be important in cancers arising from a constitutional defect in DNA repair, where they could influence treatment of children with malignancies arising from a constitutional defect in DNA damage recognition and repair. If these pathways prove to be somatically mutated in childhood malignancies, an approach that targets an acquired defect in DNA damage response pathways might increase the effects of chemotherapy without resulting in increased toxicity.

In summary, understanding DNA damage recognition and response pathways might not only help to manage rare forms of childhood cancer in children with a constitutional defect in these pathways, but also contribute to the understanding why and how childhood cancer is initiated and progresses. In addition, as these pathways are involved in the cellular response to chemotherapy, understanding these pathways might result in novel therapeutic strategies.

References


