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Review

The Fanconi anemia pathway: Insights from somatic cell genetics using DT40 cell line

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ABSTRACT

The Fanconi anemia (FA) pathway is a complex phosphorylation–ubiquitination network in the DNA damage signaling, which is still poorly understood. Defects in the "FA pathway" or in the related DNA repair proteins cause FA, a hereditary disorder that accompanies compromised DNA crosslink repair, poor hematopoetic stem cell survival, genomic instability, and cancer. For molecular dissection of the FA pathway, we have been using chicken B cell line DT40 as a model system. In this review, we will summarize our current understanding of the pathway, and discuss how studies using DT40 have contributed to this rapidly evolving field.

Contents

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1. Introduction

As part of the special issue on Fanconi anemia (FA), we would like to summarize current understanding on FA and how studies using DT40 cells have contributed to the field. DT40 is an avian B lymphocyte cell line, which has been derived from retrovirally induced lymphoma in the Bursa of Fabricius [1]. In 1991, Drs Jean Marie Buerstedde and Shunichi Takeda reported that this cell line has an extremely high capability in targeted integration of a plasmid DNA into homologous genomic locus [2]. This property is useful in making gene-disrupted cell lines, and DT40 has been exploited in a number of studies in cellular and molecular biology.

Our laboratory has been focusing on FA, a rare hereditary disorder characterized by progressive bone marrow failure, compromised genome stability, and increased incidence of cancer (for clinical aspects of FA, see "Fanconi Anemia and its Diagnosis" Auerbach, this issue) [3–6]. FA is caused by multiple genetic defects, and currently altogether 13 genes have been identified. Mutations in any of these genes result in a similar FA phenotype (except for FA-D1 and FA-N patients; they display severer symptoms), indicating that the FA gene products constitute a common biochemical network often referred to as "FA pathway".

Although we use other cell lines and systems as well, DT40 still remains our favorite model in analyzing molecular mechanisms of FA, since it is a quick and easy system to carry out "clean" genetic experiments. Gene targeting in DT40 cells normally needs screening only 20–30 clones to identify correct targeting events. They grow very fast, and are easy to handle. In contrast, cell lines from FA patients are generally difficult to grow, and even simple stable transfectants might be difficult to obtain. The RNA interference in cultured mammalian cells is certainly highly useful. However, it might not always lead to sufficient reduction of the protein expression, and the remaining protein levels may hamper definite interpretation of the data. In DT40 cells, sophisticated genetic manipulations such as conditional or double/triple knockouts have been achieved. Furthermore, we can introduce a subtle "knock-in" mutation into a locus without touching cis genetic elements. This seems highly useful for dissecting biochemical pathways. However, one potential drawback is that some antibodies against mammalian protein do not cross-react with the chicken protein. "Knock-in" of a fluorescent protein or an epitope tag could overcome this problem. Finally, DT40 is a chicken cell line, and we should be careful about the possibility that there could be some difference between human and chicken system, although fundamental molecular mechanisms are well conserved through evolution.

Based on the known structural and functional properties, the FA proteins may be classified into three groups: (1) components of the FA core complex (FANCA/B/C/E/F/G/L/M), (2) FANCD2 and FANCI, forming I–D complex, (3) breast cancer susceptibility proteins BRCA2/FANCD1, PALB2/FANCN, and BRIP1/FANCJ [5]. In addition, a few gene products have been discovered, which associate with the FA core complex (e.g. FAAP100 and FAAP24 proteins) but without known FA patients lacking these factors [5]. In Table 1, we summarized homologies between human FA genes and chicken counterparts. Fig. 1 shows chromosomal locations of the chicken FA genes as well as FA-related genes.

There is an established link between group (1) and group (2) proteins through the signal transduction pathway leading to FANCD2 monoubiquitination, while group (3) proteins are proposed to function down stream of the (1) and (2) proteins [5]. However, in our view, there is no hard evidence to support this hypothesis, and it is possible that they could function in a distinct manner to the rest of the FA proteins (see below). Therefore hereafter in this review, we will use the term "FA pathway" to indicate the FA core complex–FANCD2/FANCI axis consisting of group (1) and (2) proteins. In case we need to refer all of them including group (3) proteins, we would use the term "FA proteins".

Table 1
Chicken FA and FA-related proteins.

	Gene	Conserved motifs	Conservation to human protein[b]
Group (1)	FANCA		48/65
	FANCB[a]		42/62
	FANCC		49/65
	FANCE		39/52
	FANCF[a]		39/52
	FANCG/XRCC9	Tetratricopeptide repeats (TPR)	35/51
	FANCL	WD40, PHD finger	70/83
	FANCM/hHEF	DEAH helicase, XPF-family nuclease domain	44/60
	FAAP100		53/66
	FAAP24[a]	XPF-family nuclease domain	62/78
Group (2)	FANCD2	Monoubiquitination	56/72
	FANCI	Monoubiquitination S/TQ cluster	66/80
Group (3)	FANCD1/BRCA2	BRC repeat, OB fold	46/60
	FANCN/PALB2[a]		44/62
	FANCJ/BRIP1	DEAH helicase domain	55/70
E2	UBE2T[a]	UBC domain	65/77
Deubiquitinase	USP1		72/81

[a] We have not yet verified sequence data of these chicken genes deposited in the NCBI database.

[b] Percent identity/similarity to human homolog was assessed by BLASTP program at the NCBI website using full-length chicken amino acid sequence. In some of the alignments, there are gaps, resulting in overestimation of the actual overall identity/similarity. Thus the values should be regarded as a rough estimate.

2. FA is a disorder defective in DNA damage response/DNA repair

Genome stability is crucial for maintaining genetic integrity of the organism, and therefore all cells have elaborate systems to repair or tolerate endogenous or exogenous DNA damage. The S phase is a particularly vulnerable period to DNA damage. Presence of DNA lesion in the genome during S phase may cause arrest of DNA replication forks, which must be reinitiated to avoid fork collapse that potentially leads to lethal double-strand breaks (DSBs) or carcinogenic chromosomal rearrangements. Cells carry out this task by two basic mechanisms including (1) template switching to the intact sister chromatid using homologous recombination (HR) strategies, or (2) DNA damage bypass in which the specialized polymerases replicate past DNA lesions and fill single-stranded gaps (termed translesion synthesis, TLS). Masao Sasaki first reported in 1973 an important observation which potentially connects FA with such mechanisms: cells from FA patients exhibit highly elevated levels of chromosome aberrations upon treatment with DNA crosslinker (e.g. diepoxybutane or mitomycin C) [7]. This has led to the proposal that the basic defect in FA is somehow related to the cellular response to interstrand DNA crosslink (ICL) and its repair mechanisms [8]. Although current evidence clearly defines FA as a disorder defective in DNA damage response and/or repair, this has not been substantiated until very recently.

Probably the first concrete evidence that implicate an FA gene in DNA damage response came with the identification of *FANCD2* reported in 2001 [9]. Although FANCD2 protein has no functional motifs known to date, it responds by inducible monoubiquitination and formation of nuclear foci to the treatment with DNA damaging agents such as UV or MMC [10]. Furthermore, the FANCD2 foci