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Nuclear Localization of the Fanconi Anemia Protein FANCC Is Required for Functional Activity

To the Editor:

Fanconi anemia (FA) is an autosomal recessive disease characterized by bone marrow failure and cancer susceptibility. At least eight complementation groups for FA have been identified, and the genes corresponding to groups A, C, and G have been cloned. An understanding of the biochemical function of the FANCA, FANCC, and FANCG proteins would yield considerable insight to the cellular function of the FA pathway. Unfortunately, the three cloned FA proteins have no recognizable structural or functional motifs, and studies regarding their interaction and cellular localization in most cases have been conflicting. First, some studies show that the FANCA and FANCC protein bind in a functional complex,^{1,2} while other studies fail to detect the complex.³ Second, some studies show that the FANCC protein is localized to the

cytoplasm and nucleus,^{1,4} while other studies show that FANCC is primarily cytoplasmic.⁵

To resolve this controversy, Hoatlin et al⁶ have used confocal microscopy to determine that FANCC is localized to both cytoplasmic and nuclear compartments. We have new evidence that strongly supports this model of Hoatlin et al. We analyzed the subcellular localization of FANCC polypeptide in retrovirally infected HeLa cells by immunofluorescence microscopy (Fig 1). The wild-type FANCC protein was detected in both nucleus and cytoplasm in varying ratios (Fig 1B), confirming the results of Hoatlin et al.⁶ In most cells, the protein was predominantly localized to the nucleus. In a small percentage of cells, a strong cytoplasmic localization of FANCC was also detected. An examination of the cell morphology and DAPI staining showed that these cells expressing cytoplasmic FANCC were

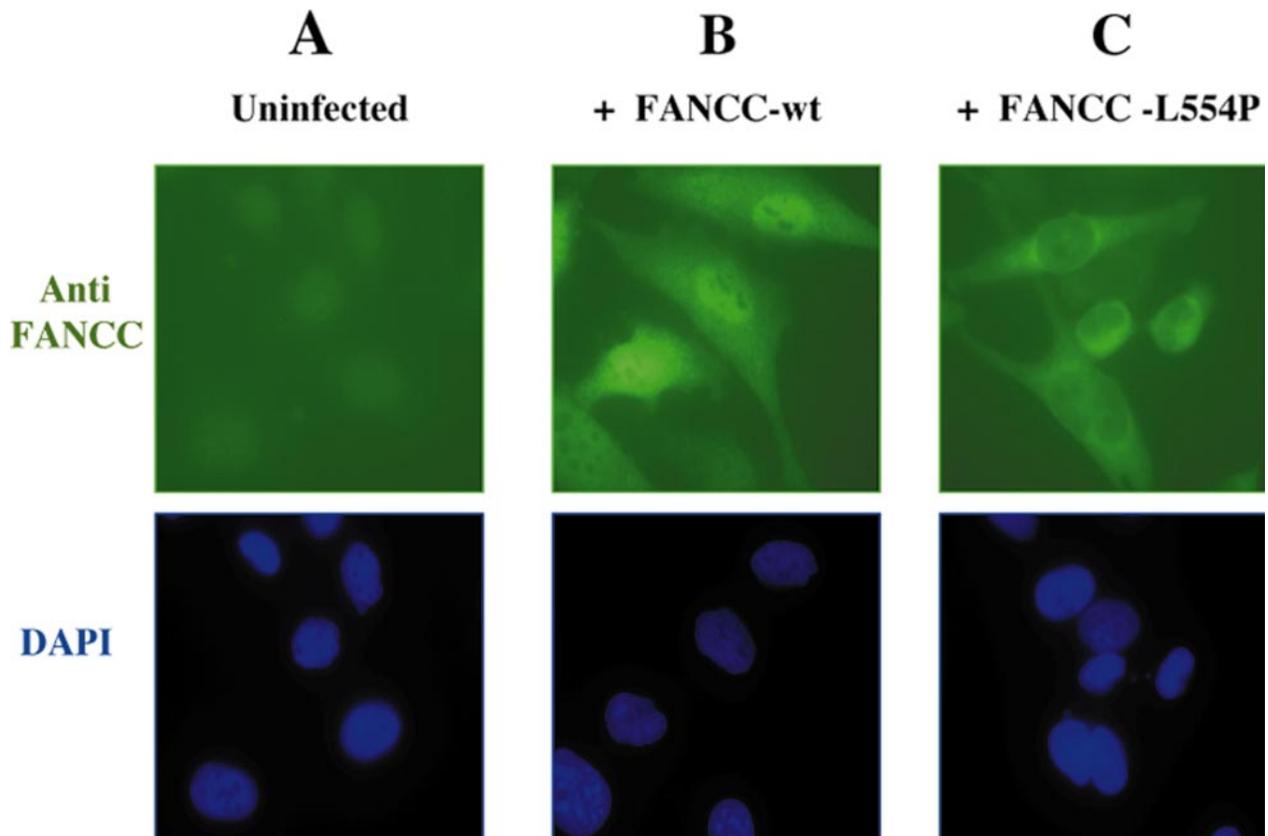


Fig 1. Cellular localization of the wild-type FANCC protein and the L554P mutant. (A) The HeLa cell line expresses low levels of endogenous FANCC protein that is undetectable by anti-FANCC immunofluorescence. HeLa cells were infected with retroviral supernatants, pMMP-FANCC (B) or pMMP-FANCC-L554P (C). Pools of infected cells were stained with anti FANCC and the DNA-specific dye, DAPI (4',6-diamidino-2-phenylindole) (bottom images) and analyzed by immunofluorescence, as previously described.⁴

undergoing mitosis or had just completed cell division, suggesting that localization may be cell-cycle dependent.

Mutations in FA patients represent an important resource for understanding both the function of the FA proteins and the molecular basis of the disease. For instance, the FA patient-derived FANCC-L554P mutant, which has a leucine to proline mutation at amino acid 554, fails to complement the mitomycin-C sensitivity of FA-C cell lines⁷ and is defective in FANCA binding.¹ To evaluate the effect of the L554P mutation on the subcellular localization of the protein, we analyzed retrovirally infected HeLa cells by indirect immunofluorescence. Interestingly, the FANCC-L554P mutant, unlike the wild-type protein, was only observed in the cytoplasm of retrovirally infected HeLa cells (Fig 1C).

On the basis of these results, we conclude that the wild-type FANCC protein is normally localized in both nucleus and cytoplasm. The absence of nuclear accumulation of the FANCC-L554P protein allows several conclusions. First, the nuclear localization of wild-type FANCC is not merely caused by the protein overexpression. Second, the functional activity of the FANCC protein requires nuclear localization. The FANCC-L554P mutation results in cytoplasmic localization and abolishes function. The missense mutation is likely to disrupt a domain of the protein required for its functional activity. The COOH-terminus domain of FANCC may be required for binding to FANCA or other protein subunits of the FA complex or for localization or stabilization of the protein in the nucleus.

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Evidence for Continuous Basal Generation of Gc-MAF: Absence in Infantile Osteopetrosis and Restoration After Bone Marrow Transplant

To the Editor:

Recent studies by Yamamoto et al¹ and Benis and Schneider² in *Blood* suggest that Gc-globulin (also known as vitamin D-binding protein) may have an important role in macrophage activation and osteoclast differentiation from monocytes and so may control bone morphogenesis and remodeling.¹⁻⁵ Deglycosylation of Gc-globulin (removal of galactose and sialic acid from the trisaccharide leaving N-acetyl-galactosamine [GalNAc]) produces a potent macrophage-activating factor (Gc-MAF).¹ In osteopetrotic rat and mice models^{4,5} and in a single human study,¹ indirect data suggest a defect in lysophospholipid-inducible Gc-MAF, although direct estimation of this factor in normal and disease state has never been performed.

In this report, we extend earlier observations by showing a basal level of Gc-MAF generation in normal healthy human subjects, its absence in a patient with infantile autosomal recessive osteopetrosis (IOP), and suppression in IOP carriers. The patient, a female infant of related (second cousin) South Asian parents, presented with seizures on the eighth day of life. Investigation showed severe hypocalcemia (plasma calcium, 1.25 mmol/L; albumin 36 g/L), with elevated parathyroid hormone level (208 pg/L reference, 10 to 54 pg/mL). Bone marrow aspirate and biopsy were consistent with a diagnosis of osteopetrosis. The extended family was highly consanguineous, with several mar-

riages between cousins, and a history of osteopetrosis in two distant cousins. A skeletal survey demonstrated uniformly dense bones with associated metaphyseal lucent bands in keeping with osteopetrosis. The father was a genotypical HLA match, and the patient underwent a conditioned unmanipulated bone marrow transplant at the age of 10 months. By day 28 post bone marrow transplant, she had an increasing lymphocyte count with serum Ca²⁺ 2.29 mmol/L, phosphate 1.27 mmol/L, and albumin 36 g/L. Molecular DNA studies confirmed engrafting.

The measurement of exposed N-acetyl-galactosamine (GalNAc) component of Gc-MAF was performed by lectin-based immunoassay using a combination of antibody monospecific to Gc-globulin protein (Diasorin, Berkshire, UK), and Helix pomatia lectin conjugated to horseradish peroxidase (HRP) (Sigma, Dorset, UK), which specifically binds to GalNAc. The specificity and reproducibility of the assay used was confirmed using appropriate positive and negative controls.

The rates of generation of the factor for the patient, her parents, and for male control subjects (n = 9) are shown in Fig 1. All control subjects showed basal noninduced generation of Gc-MAF when lymphocytes were incubated with the plasma Gc-globulin (ΔOD_{620} range, 84 to 180; mean \pm SD, 95 \pm 18). The patient showed complete lack of generation of Gc-MAF pretransplantation, despite prolonged incubation of lymphocytes and plasma, lasting 16 hours. After transplan-