Oligomerization of Ferroportin and the Mechanism of Autosomal Dominance in Ferroportin Disease

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Introduction: Ferroportin (Fpn)—the only known cellular iron-export protein—plays a critical role in iron homeostasis by mediating iron export from macrophages and enterocytes. Rare, loss-of-function mutations in human Fpn (e.g. V160del) cause classical ferroportin disease, characterized by macrophage iron loading and iron-restricted anemia. Ferroportin disease displays an autosomal–dominant inheritance pattern, and all classical ferroportin disease-associated mutations are missense.

Hypothesis: We hypothesize that the autosomal–dominant inheritance of classical ferroportin disease is exerted by a dominant–negative effect resulting from oligomerization of wildtype and mutant Fpn proteins to form nonfunctional complexes.

Methods: We have examined the functional activity of wildtype (wt) and mutant human Fpn co-expressed in RNA-injected *Xenopus* oocytes. We measured ^{55}Fe efflux from control oocytes, or oocytes expressing wtFpn–GFP or V160del–GFP fusion proteins in the presence or absence of a co-expressed wt-Fpn–mCherry fusion protein. We determined the RNA dose producing half-maximal ^{55}Fe efflux activity (one “unit”) to be 7.5 ng for wtFpn–GFP RNA and 13 ng for wtFpn–mCherry RNA. Since V160del is nonfunctional, we calibrated the plasma-membrane fluorescence of V160del–GFP against that of a unit of wtFpn–GFP, to estimate one unit of V160del–GFP RNA to be 30 ng. We set critical significance level, $\alpha = 0.01$.

Results: ^{55}Fe efflux activity in oocytes co-expressing wtFpn–GFP and wtFpn–mCherry constructs was additive. Expression of V160del–GFP did not stimulate ^{55}Fe efflux activity compared with control, whereas co-expression of the mutant along with wtFpn–mCherry virtually abolished the ^{55}Fe efflux activity observed for wtFpn–mCherry alone (2-way AVOVA: interaction, $P < 0.001$). In a second study, progressively increasing amounts of V160del–GFP RNA (in the range 0.1–100 ng) inhibited the ^{55}Fe efflux activity in oocytes co-expressing one unit of wtFpn–mCherry in a dose-dependent fashion. The data (activity as a function of V160del RNA amount) were fit by a 4-parameter logistic function ($r^2 = 0.92$, $P = 0.002$) in which half-maximal inhibition was observed at estimated dose (± SE) of 29 ± 7 ng V160del RNA.

Conclusions: Our results provide evidence of a dominant–negative effect of the V160del mutant on Fpn activity and support the notion that oligomerization of Fpn underlies the autosomal dominance of ferroportin disease.

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