Action of "correctors" of human ΔF508 CFTR on the mutant protein from other species

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The mutation (ΔF508) responsible for most cystic fibrosis causes the protein product (CFTR) of the mutant gene to be misassembled and thermally unstable at body temperature. Some new investigational drugs found to improve assembly fail to restore thermal stability. Here we investigated the influence of one of these compounds, VX-809 on ΔF508 versions of CFTR from several non-human species with varying degrees of thermal stability and found that the assembly of all was further improved, indicating that biosynthetic assembly and thermal stability both need to be restored for effective correction of the disease-causing defect.

The ΔF508 mutation that causes most cystic fibrosis impairs the biosynthetic and thermal stability of the ion channel protein. Multiple strategies and experimental methods have been applied to study the details of the altered structure and dynamics of the mutant protein and aid in the development of novel corrective manipulations[3]. As one of these strategies, we have explored the variable effects of the mutation on the ΔF508 versions of CFTRs of several mammalian and non-mammalian species other than human[1,2]. The relative insensitivity of the latter group was found to be primarily due to the presence of proline residues (in addition to I539T) in flexible regions of NBD1 including the Regulatory Insertion (RI), the Structurally Diverse Region (SDR) and the γ-PO_4 switch loop. Introduction of proline into the corresponding positions of human ΔF508 CFTR provided a similar level of thermal stability of channel activity. We now have employed ΔF508 CFTRs from different species with a range of different sensitivities to the destabilizing influence of the F508 deletion to gain some insight into the poorly understood mode of action of small molecule "correctors" of the defect. These compounds, discovered in high throughput cell based screens, promote its maturation and traffic to the cell surface but long term efficacy is quite limited, possibly because they do not restore thermodynamic stability. Since this is the property of the mutant protein that varies among different species, we reasoned that the responses to a corrector might provide an indication of whether it acted by stabilization or by some other indirect mechanism.

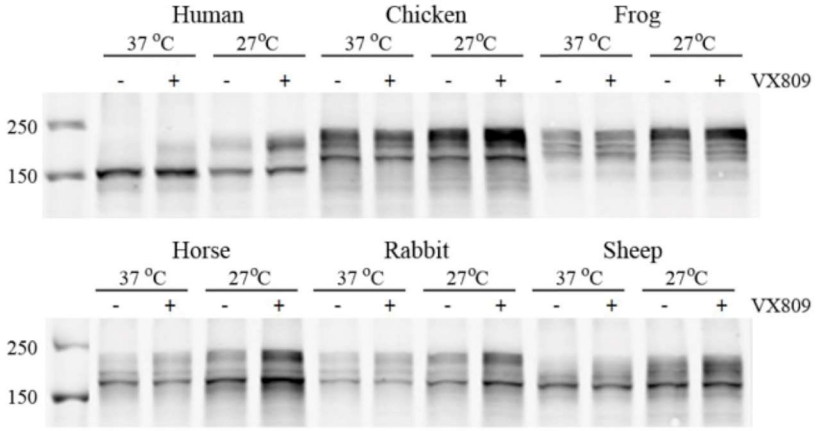

Figure 1. Western blots of ΔF508 CFTRs of the species indicated expressed in HEK-293 cells with (+) and without (-) exposure to 3 mM VX-809 for 20 h at the temperatures indicated. 20 mg of total cell lysate protein was loaded. In all cases, exposure to the compound increased maturation (conversion to the higher molecular weight forms) at both temperatures, indicating it acts additively with the low temperature influence.

Using the investigational drug, VX-809, one of the most effective "correctors" yet discovered[4] we observed that it resulted in a further increment in maturation of ΔF508 CFTR of all species regardless of the degree of maturation in mammalian cells at 37°C not exposed to the compound (Fig 1). This result suggests that the compound acts by a means other than by increasing thermodynamic stability which appears also to be true of

other existing "correctors" and emphasizes the need to identify additional compounds that may have this capability.

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