C1 Complement mediates human cord blood serum derived APP α-secretase cleavage activity in vitro

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Abstract
Alzheimer’s disease (AD) is the leading cause of dementia in the elderly. In healthy individual amyloid precursor protein (APP) is cleaved by α-secretase generating sAPPα. However, in the neurodegenerative environment of AD patients, APP peptides of either 40 or 42 residues are generated by increased beta and gamma secretase activity. Human umbilical cord blood cells (UCBC) have proven useful as potential immunomodulatory therapies in various models of neurodegenerative diseases. Our study investigated the impact of UCBS on modulation of sAPPα production. Heat-activated UCBS has significantly promoted sAPPα production indicating presence of heat sensitive α-secretase in CBS. Using LC-MS/MS, we identified the subunits of C1 complex (C1q, C1r and C1s) and a-2-macroglobulin showed significantly greater levels in aCBSF compared with AgBSF. Specifically, C1a markedly increased sAPPα and αCTF production, whereas C1q alone only minimally increased and C3 did not increase sAPPα production in the absence of sera. Furthermore, C1q markedly increased sAPPα and αCTF, while decreasing Aβ, in CHO/APPwt cells cultured in the presence of whole sera. These results confirm that APP α-secretase activity in human blood serum is mediated by C1 opening a potential modality of therapeutic for the future of AD.

Results

1. APP α-secretase activity in CBS
CHO/APPwt cells were treated with 2% CBS for different time. The result indicated the time-dependent production of sAPPα (Fig. 1a). Similarly, CHO/APPwt cells indicated dose-dependent production of sAPPα after treating with different concentrations of CBS (Fig. 1b). CHO/APPwt cells treated with 2% whole or heat-inactivated (56°C) CBS, AgBS or or their purled fraction (αCBSF, αAgBSF) all showed decreased sAPPα levels (Fig. 1c, Fig. 1d).

2. APP α-secretase activity in CBS is independent of enzymatic activity complement C3b
We identified 142 the major proteins with different expression in fraction αCBSF and αAgBSF. Several of the major proteins identified that most likely to exhibit α-secretase activity are shown in Fig. 2a. We first investigated whether depletion of C3 could limit the activity of CBS α-secretase, and found that A3b, a C3b inhibitor, did not change sAPPα production after mixing with 1.6% whole (Fig. 2b) as well as C3 depleted (Fig. 2c) CBS at different dose in CHO/APPwt. Inactivation of C3b using inhibitor of C3b/C3b) does not increased sAPPα production markedly (Fig. 3d).

3. APP α-secretase activity in CBS is mediated in part by complement C1 complex
To determine if α-secretase activity in CBS is mediated by C1 complex, CHO/APPwt cells were treated with 1 or 2% CBS that supplemented with C1 inhibitor, and the results showed that the sAPPα production was markedly reduced in a dose-dependent fashion (Fig. 3a, Fig. 3b). In order to further confirm that C1 complex mediates α-secretase activity in CBS, we determined the effect of purified human

Conclusion
Collectively, our results indicate that CBS contains proteins that promote α-secretase like enzymatic activity. LC-MS/MS analysis in CBSF and AgBSF revealed the presence of 142 proteins of which 1 subunits and alpha-2-macroglobulin showed significantly greater levels in CBSF compared with αAgBSF. Further study showed C1 subunits can enhance sAPPα production and αCTF reduction in cell culture condition.