Development of *in vitro* assays to detect BACE-dependent shedding of Tmem27

Iryna Voytyuk, supervised by Brian J. Hrupka and Diederik Moechars

Janssen Research & Development, A Division of Janssen Pharmaceutica NV, Beerse, Belgium

**Introduction**

Amyloid-beta (Ab) plaques are one of the hallmarks of Alzheimer’s disease (AD). They are comprised of Ab peptides, which are produced through cleavage of the amyloidogenic precursor protein (APP) by beta-secretase (BACE1) and gamma-secretase (GSI) (De Strooper B 2010). AD is rapidly becoming a public health challenge due to the increasing worldwide burden of this disease, as well as its economic impact and the increasing global life expectancy. Therefore, it is an urgent need for global prevention, diagnostic, and therapeutic measures for this devastating disease.

One of the promising AD drug targets is the β-site amyloid precursor protein (APP) by its N-terminal and C-terminal cleavage (Esterhazy 2011). γ-secretase is only a small part of BACE1 protease, which has its own secretome and is highly expressed in other tissues (Stutzer, 2013, Rochin 2013). In the pancreatic β-cells, BACE1-dependent cleavage of many membrane proteins is essential for various physiological processes like proliferation, maintenance of insulin secretion, and cell survival (De Strooper B 2010; Zhou, 2012). BACE1-dependent cleavage of many membrane proteins is essential for various physiological processes like proliferation, maintenance of insulin secretion, and cell survival (De Strooper B 2010; Zhou, 2012). BACE1-dependent cleavage of many membrane proteins is essential for various physiological processes like proliferation, maintenance of insulin secretion, and cell survival (De Strooper B 2010; Zhou, 2012). BACE1-dependent cleavage of many membrane proteins is essential for various physiological processes like proliferation, maintenance of insulin secretion, and cell survival (De Strooper B 2010; Zhou, 2012).

**Materials and methods**

Transfections were performed using Lipofectamine 2000 (Invitrogen). For this purpose five different constructs were designed: (n)Flag-(TMEM27-HA(c)), (n)3xFlag-(n)HA-(n)TMEM27-(c), (n)HA-(c)TMEM27-(n)Flag (c), (n)Myc-(n)TMEM27-(c), (n)V5-(n)TMEM27-(c). When inhibition experiments were conducted, the transfection medium contained 0.5 mM BACE1 inhibitor (BACEi) or γ-secretase inhibitor (GSI) dissolved in DMSO using DMSO as control.

**Stable cell line development**

Stable cell lines expressing Tmem27 were generated through the transfection of Min6 cells with plasmids. A second generation of stably transfected Min6 cells (n)HA-(c)TMEM27-(n)Flag was obtained by cloning into a minigene. This clone is used in the future to test potential BACE1 inhibitors for their enzyme specificity. From the stable clones tested, Clone 6 showed the most promise as a consistent expresser of the tagged Tmem27, with easily detectable levels by IIF and IF.

**Results**

Figure 1: Expression of tagged Tmem27 in the stable cell lines stably transfected with (n)Flag-(c)TMEM27-(n)HA. 

Figure 2: Detection and characterization of min clones stably transfected with (n)V5-(c)TMEM27-(n)HA construct. 

**Conclusions**

Translation of V5-Tmem27-HA construct into Min6 cells allowed efficient detection of Tmem27 by its N-terminal and C-terminal HA tags. Cleavage of overexpressed Tmem27 is mediated by BACE2 and gamma secretase in the Min6 cells. Because Tmem27 is a BACE2 substrate, stably expressed V5-Tmem27-HA results in a consistent and reliable cellular model to test potential BACE1 inhibitors for their enzyme specificity. From the stable clones tested, Clone 6 showed the most promise as a consistent expresser of the tagged Tmem27, with easily detectable levels by IIF and IF.

**Implications**

In the future, quantitative detection of substrate cleavage in this cellular assay will allow for biochemical assays in testing potential BACE1 inhibitor compounds. Many new BACE1 inhibitors have now entered clinical trials, but their initial testing mostly focused on APP cleavage alone, so it is difficult to assess whether the compounds will have any on-target and off-target effects on patients. Such effects need to be monitored carefully and back up programs need to be established taking this potential liability into account. Min6 stable cell lines expressing Tmem27 is a cellular model suitable for future drug screening, bringing us one step closer to a specific BACE1 inhibitor sought for treatment of Alzheimer’s disease.

**Acknowledgments**

This work was funded by and carried out at Janssen Pharmaceutica, Beerse, Belgium. I would like to thank my supervisors Brian J Hrupka and Diederik Moechars, as well as Gian de Vos Kerkhoven for input on experimental procedures and scientific advise. We also thank Janssen Research and Development San Diego, Department of Neurological Disorders for providing the Min6 cell line and Stephen Masure for Tmem27 design construct, as well as Dr. Bart de Snooer for helpful discussion.

**Literature cited**

