Targeting novel Integrative Nuclear FGFR1 Signaling (INFS) reconstitutes active neurogenesis in adult brain.

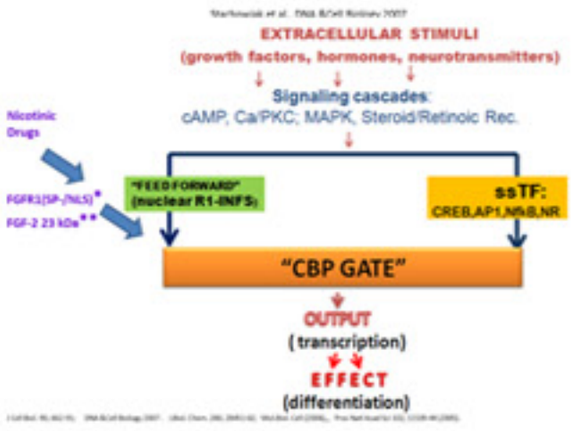
Sridhar Narla S[1]., Ilona Klejbor[2], Barbara Birkaya[1], Yu-Wei Lee[1], Janusz Morys[2], Ewa K. Stachowiak[1], Dorota Prokop[1], Merouane Bencherif[3], and Michal K. Stachowiak[1]
[1]Department of Pathology and Anatomical Sciences, State University of New York at Buffalo, Buffalo, NY, USA, [2]Department of Anatomy and Neurobiology, Medical University of Gdansk, Poland and [3]Preclinical Research, Targacept, Inc., Winston-Salem, NC

I

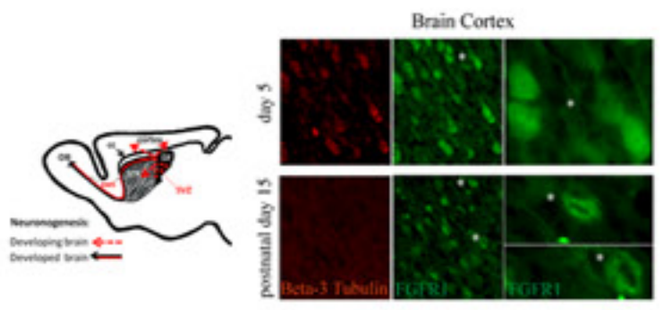
Introduction

Reactivation of endogenous neurogenesis in the adult brain or spinal cord holds the key for treatment of CNS injuries as well as neurodegenerative disorders, which are major healthcare issues for the world's aging population. We have previously shown that activation of developmental Integrative Nuclear FGFR1 Signaling (INFS), via gene transfection, reactivates neurogenesis in the adult brain by promoting neuronal differentiation of brain Neural Stem/Progenitor Cells (NS/PC). In the present study, we report that targeting the α7 nicotinic acetylcholine receptors (α7nAChR) with a specific TC-7020 agonist leads to a robust accumulation of endogenous FGFR1 in the cell nucleus. Nuclear FGFR1 accumulation is accompanied by an inhibition of proliferation of NS/PC in subventricular zone (SVZ) and by the generation of new neurons. Neuronal differentiation is observed in different regions of the adult mouse brain, including: (1) βIII Tubulin-expressing cortical neurons, (2) calretinin expressing hippocampal neurons and (3) cells in substantia nigra expressing predopaminergic Nurr1+ phenotype. Furthermore, we show that *in vitro* stimulation of neural stem/progenitor cells with α7nAChR agonist directly activates INFS and neuronal-like differentiation. TC-7020 stimulation of the βIII Tubulin gene is accompanied by increased binding of FGFR1, CBP and RNA Polymerase II to a Nur77 targeted promoter region. TC-7020 augments Nur77 dependent activation of NBRE (Nerve Growth Factor inducible-B protein Responsive Element) indicating that α7nAChR upregulation of βIII-Tubulin involves neurogenic FGFR1-Nur signaling. The reactivation of INFS and neurogenesis in adult brain by the α7nAChR agonist may offer new strategy to treat brain injuries, neurodegenerative and neurodevelopmental diseases.

Fig. 1 Integrative Nuclear FGFR1 Signaling (INFS) is a common "Feed-Forward- And-Gate" module that transmits diverse developmental signals.



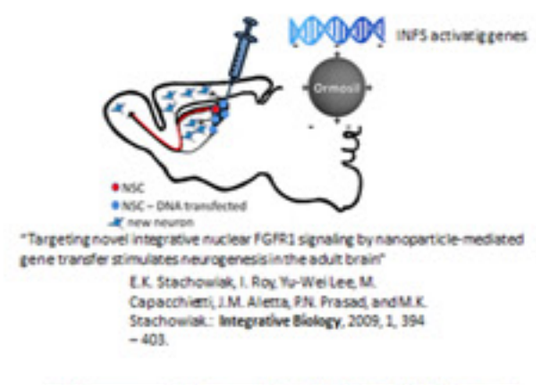
The INFS is active in developing cortical neurons and inactive in mature brain



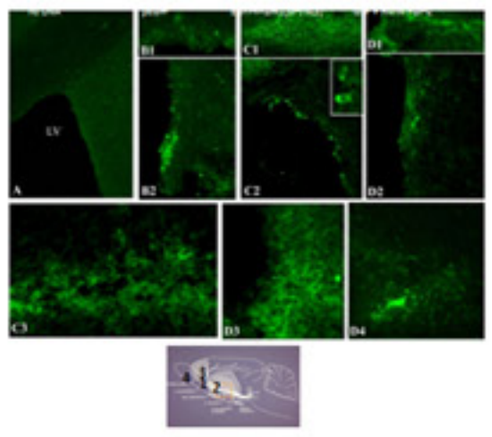
At postnatal day 5 brain cortex contains βIII-tubulin expressing immature neurons.
In those cells FGFR1 is present in the nucleus.
At postnatal 15 FGFR1 is predominantly cytoplasmic. βIII-tubulin neurons are absent from the cor

II

Direct activation of INFS by transfection of nuclear FGFR1(SP-/NLS) or 23 kDa FGF-2, which activates endogenous nuclear FGFR1, stimulates in vivo neuronogenesis.

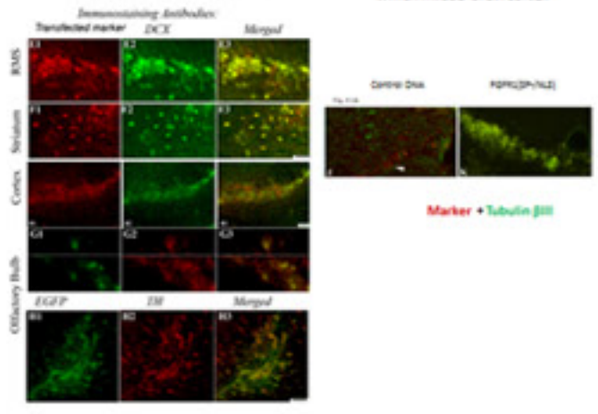


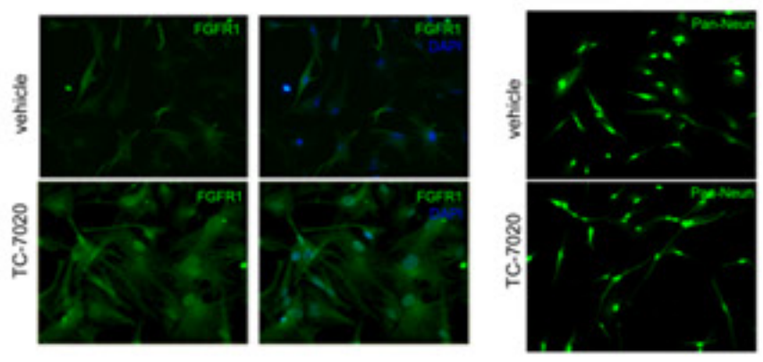
"Targeting novel integrative nuclear FGFR1 signaling by nanoparticle-mediated gene transfer stimulates neurogenesis in the adult brain"
E.K. Stachowiak, I. Roy, Yu-Wei Lee, M. Capacchietti, J.M. Alerta, P.N. Prasad, and M.K. Stachowiak.: **Integrative Biology**, 2009, 1, 394 – 403.



The majority of FGFR1(SP-/NLS) transfected cells express doublecortin (DCX)

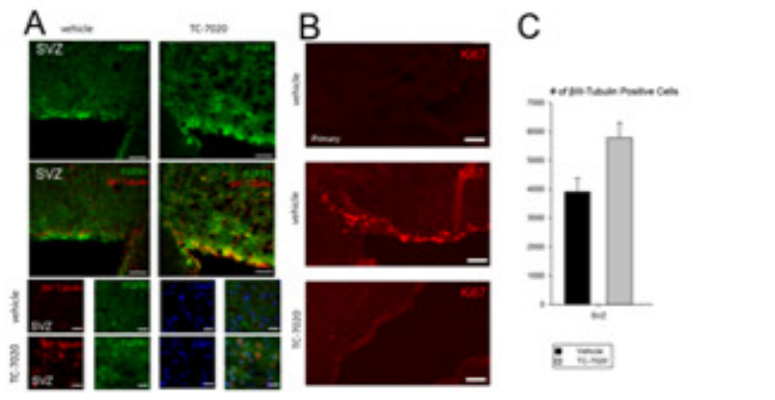
FGFR1(SP-/NLS) transfected cells differentiate to **Tubulin βIII** + neurons which invade brain cortex



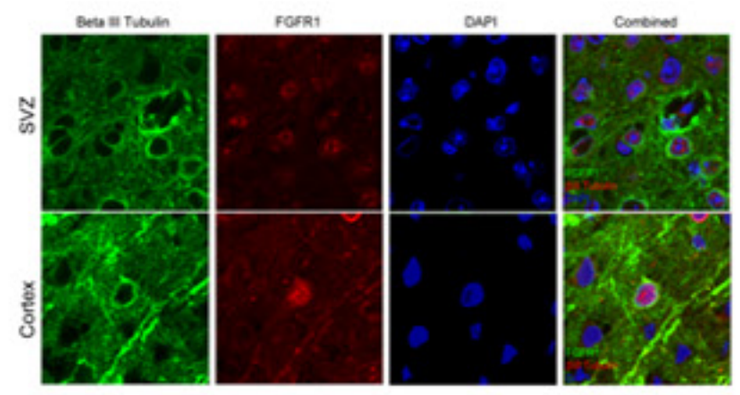


III

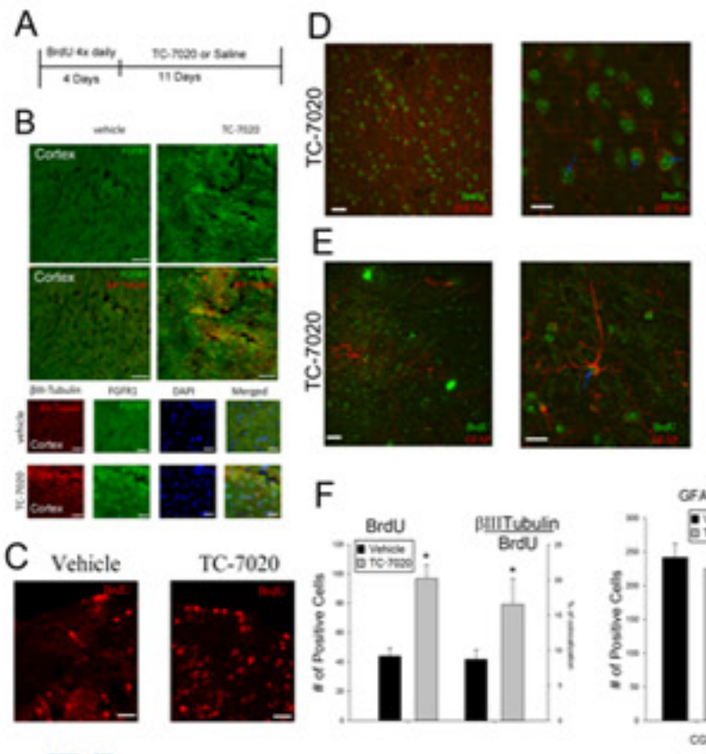
TC-7020 activate INFS and reduces Proliferation in SVZ in adult mouse brain



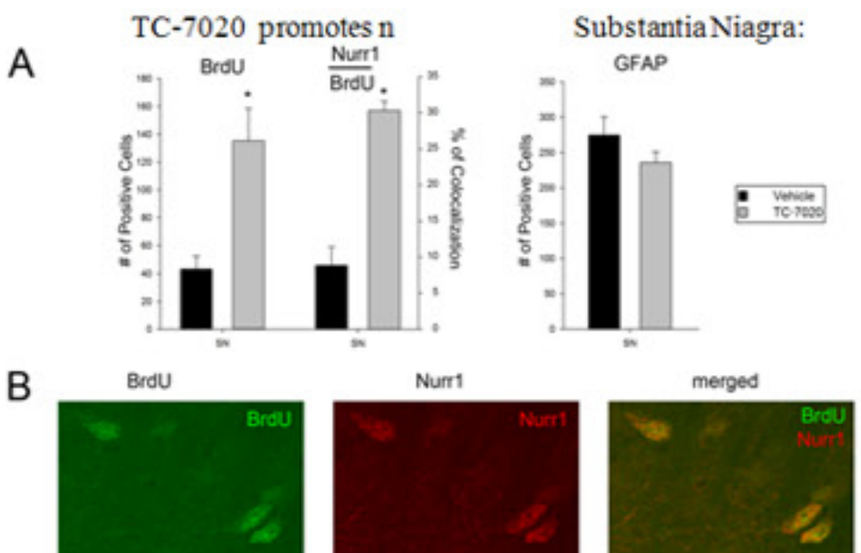
Nuclear FGFR1 Colocalizes with Immature neuronal marker in cortex and SVZ



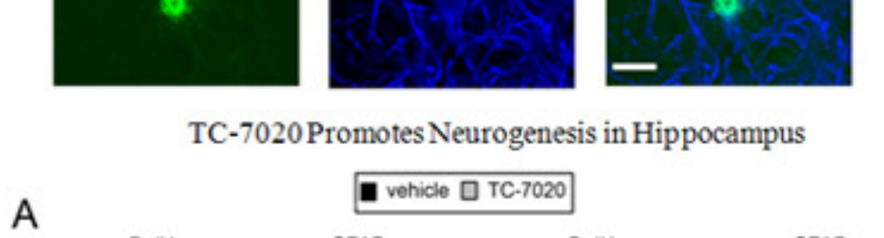
TC-7020 Promotes Neurogenesis, in cingulate cortex



IV



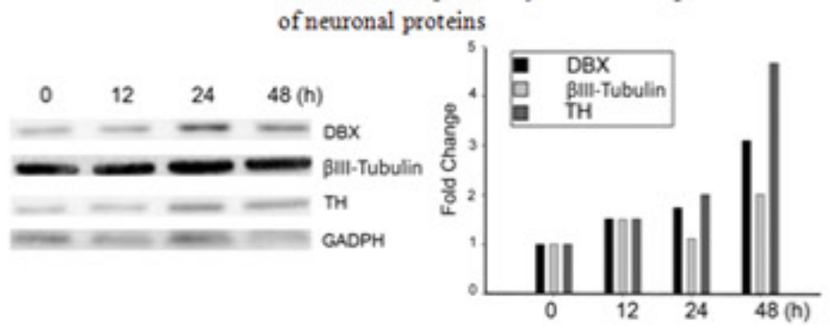
TC-7020 Promotes Neurogenesis in Hippocampus



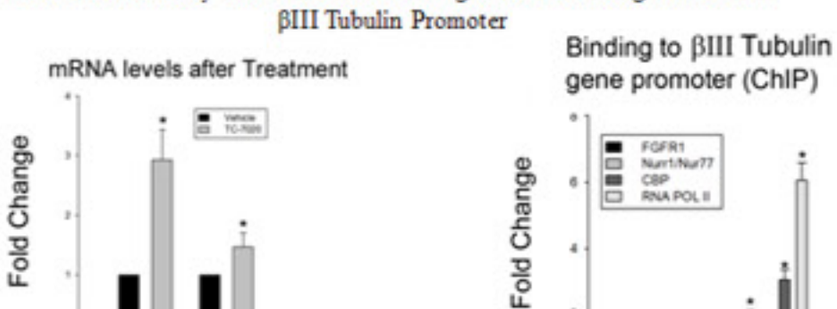
TC-7020 Causes nuclear accumulation of FGFR1 in neuroblastoma

V

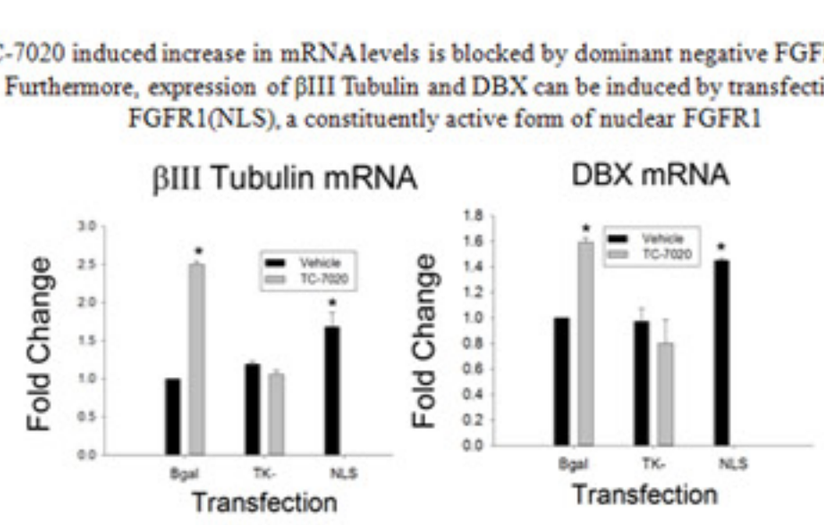
Nuclear accumulation of FGFR1 is accompanied by increased expression of neuronal proteins



TC-7020 Treatement causes increase in βIII Tubulin and Double Cortin mRNA. ChIP assay shows increaed binding of FGFR1 along with CBP in βIII Tubulin Promoter



TC-7020 induced increase in mRNA levels is blocked by dominant negative FGFR1(TK-). Furthermore, expression of βIII Tubulin and DBX can be induced by transfection of FGFR1(NLS), a constituently active form of nuclear FGFR1



Conclusion: The recent study shows that targeting specifically α7nAChR reactivates the developmental INFS module along with the post-mitotic neuronal development in adult brain SVZ and hippocampus. Generation of new neurons is also observed in the brain cortex and SN, where little neurogenesis occurs in the mature brain. Thus, the TC-7020 induction of new neurons that extends to the brain cortex, hippocampus or SN, re-emphasizes the possibility of latent neurogenesis in these brain regions. Activation of cortical neurogenesis by TC-7020 raises hope for new treatments of cortical injuries, stroke, and neurodegeneration in Alzheimer's or Huntington's diseases. Similarly TC-7020 activation of hippocampal neurogenesis could be applicable to treatments of dementias resulting from the loss of hippocampal neurons. Reactivation of neurogenesis in the adult brain by targeting the INFS with α7AChR agonist may represent an important step towards these therapeutic goals.

Activation of Developmental Nuclear FGFR1 Signaling and Neurogenesis in Adult Brain by α7 Nicotinic Receptor agonist. Accepted in Stem Cell Translational Medicine