Cell and Tissue Research (2022) 387:9–12
https://doi.org/10.1007/s00441-021-03571-z

EDITORIAL

To celebrate the 80th birthday of Klaus Unsicker: discovery of a new growth factor and studies on the effects of growth factors on adrenal chromaffin cells and neurons

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Published online: 3 January 2022

Klaus Unsicker published first studies on growth factors investigating the effects of nerve growth factor (NGF) on adrenal chromaffin cells in 1977–1978 (Unsicker et al. 1978a, b). NGF was the first growth factor that was discovered by Rita Levi-Montalcini and biochemically characterized by Stanley Cohen. They were awarded the Nobel Prize in physiology or medicine in 1986 "for their discoveries of growth factors". Now we know that NGF is the type member of the family of neurotrophins consisting of NGF, the best-studied neurotrophin, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4). They regulate the proliferation, differentiation, survival, adult maintenance and plasticity of several types of different neurons. They show also prominent effects on non-neuronal cells. They regulate development, physiology and maintenance of the nervous system by binding to and activating mainly two different receptor systems. They activate either transmembrane receptor tyrosine kinases that are members of the tropomyosin related kinase (Trk) family or the p75 neurotrophin receptor ($p75^{NTR}$) that is the member of the tumour necrosis factor receptor superfamily.

In these early pioneering studies, Klaus investigated explants and enzyme-dispersed cells of adrenal medulla from early postnatal rats and found that NGF caused a striking increase in the number of axons growing out from explants. Then, he investigated the role of cyclic AMP, dexamethasone and cholera toxin on NGF effects on chromaffin cells (Unsicker et al. 1980) and then in 1982 published a paper where he for the first time demonstrated that in addition to the induction of neurite outgrowth, NGF has also survival-promoting effect on early postnatal rat adrenal medullary cells (Unsicker et al. 1982). Having shown in the earlier studies that adrenal chromaffin cells from early postnatal rats maintained in culture grow neuritic processes and survive better in the presence of NGF, he now in collaboration with Silvio Varon investigated the effects of just discovered ciliary neurotrophic factor (CNTF; at that time called neuronotrophic factor) (Unsicker et al. 1985). They found that CNTF has neuritogenic effects of adrenal chromaffin cells and shows some additive effects when the cells were treated with NGF and CNTF together and then they

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report a CNTF-like activity in a neuroblastoma cell line (Heymanns and Unsicker et al. 1987). Soon after the discovery of BDNF, NT-3 and NT-4, Klaus investigated the developmental roles of these novel neurotrophic factors and in 1998 published a seminal paper demonstrating that NT-4 from chromaffin cells operates through TrkB receptors to regulate development and maintenance of the preganglionic innervation of the adrenal medulla (Schober et al. 1998). Few years later analysing NT-4 deficient mice, he found that these mice have profound structural and chemical deficits in sympathetic ganglia and their preganglionic innervation (Roosen et al. 2001). Studies on neurotrophins are culminating in a series of very important publications on the role of neurotrophin receptors. Firstly, Klaus and his team made an exciting finding showing that haploinsufficiencies of the BDNF receptor TrkB and/or NT-3 receptor TrkC caused a reduction in number of substantia nigra (SN) neurons in aged mice, which is accompanied by a reduced density in striatal fibres of dopamine neurons. These aged mutant mice, in contrast to wild-type littermates, display an accumulation of α-synuclein in the remaining dopamine neurons in the SN (von Bohlen et al. 2005). Loss of SN neurons and aggregation of α-synuclein in these neurons are hallmarks of Parkinson's disease. Although investigations on the role of neurotrophic factors and α-synuclein have been in the centre of attention during last two decades, the contribution of BDNF and NT-3 receptors in this process has not been sufficiently studied. Secondly, he asked whether there is a link between impaired hippocampal synaptic plasticity, altered spines and TrkB receptors and performed a quantitative analysis of spine densities and spine length in the hippocampal area CA1 in mice where TrkB was conditionally deleted. They found that mutant mice exhibit specific reductions in spine densities and a significant increase in spine length of dendrites in this brain area strongly suggesting that TrkB functions in structural remodelling of hippocampal dendritic spines (von Bohlen et al. 2006). In the third study, they investigated the significance of TrkB and TrkC for the maintenance of dendritic spines analysing hippocampi of TrkB and TrkC knockout mice. They found that deletion of one allele of TrkB, but not TrkC, significantly reduces spine densities of CA1 pyramidal neurons in mice, demonstrating that only TrkB receptors are necessary for the maintenance of hippocampal spines (von Bohlen et al. 2008).

One of the exciting areas of Klaus Unsicker's research concerns transforming growth factor beta superfamily members and their role in neuronal development and disease. TGF-beta (TGFβ) superfamily is the largest family of secreted growth factors with over 35 ligands in humans. TGFβ family growth factors are key regulators of *embryonic development* and adult tissue *homeostasis* and in case of misregulation may lead to a range of human diseases. Typical members of the TGFβ family are TGFβ1-3, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and activins that signal into the cell via two groups of transmembrane serine-threonine kinase receptors, so-called type I and type II receptors, that are activated by binding of the members of the TGFβ superfamily of ligands.

This superfamily also contains two distant groups of growth factors. One is the glial cell line–derived family of neurotrophic factors family consisting of four members: glial cell line-derived neurotrophic factor (GDNF), neurturin (NRTN), artemin (ARTN) and persephin (PSPN). Differently from traditional members of the TGFβ superfamily, GDNF family members use a completely different receptor system. They bind to ligand specificity determining GDNF family receptor alpha (GFRα) proteins and signal to the cells via transmembrane receptor tyrosine kinase RET. GDNF family members have multiple effects on different neuronal populations but have also very profound effects outside the nervous system.

Another stranger in the TGFβ superfamily is growth differentiation factor 15 (GDF15), a stress response protein that similarly to GDNF a distant member of TGFβ superfamily. It binds to GDNF family receptor alike (GFRAL) and similarly to GDNF family members signals through the receptor tyrosine kinase RET. GDF15 serum levels are increased in stress and disease rendering weight loss, anorexia and changes in the metabolism. Current data indicate that GDF15 activates GFRAL-RET signalling solely in the hindbrain in the area postrema (AP) neurons resulting in reduced food intake and loss of body weight. Despite recent progress, there are still many open questions. The expression of GFRAL and RET proteins, especially in the human brain, is poorly studied.

TGFβ family members have a great potential in biotechnology and also as drug candidates. GDNF and related factor NRTN have been tested in phase II clinical trials in Parkinson's disease patients, and very recent data on GDNF are promising. GDF15 is one of the hottest molecules that has potential for body weight control, but its highly elevated levels in cancer cachexia patients predict that its inhibition can be also therapeutically very valuable. In a pioneering paper published in 1995 in EMBO Journal, Klaus and his team demonstrated that TGFβs, activin A and GDNF supported the survival of cultured dopamine neurons and that the effect was not mediated by glia (Krieglstein et al. 1995). They were also the first to show that at least two different splice isoforms of GDNF are synthesized that are selectively expressed in the developing brain (Suter-Crazzolara and Unsicker 1994). Now we know that these isoforms also encode two different GDNF protein isoforms that are secreted differently. I think one of the highlights of Klaus Unsicker's research in growth factor field is the discovery demonstrating that GDNF requires TGFβ for its full neurotrophic activity. In a seminal paper published in

1998 in the Journal of Neuroscience together with his wife Kerstin Krieglstein, they demonstrated using cultured neurons, including peripheral autonomic, sensory and dopamine neurons, that GDNF is not trophically active unless supplemented with TGFβ (Krieglstein et al. 1998, 2000). Soon they found that also in vivo effects of GDNF in animal models of Parkinson's disease require the presence of TGFβ. The highlight of these studies was published in the Journal of Cell Biology in 2002. In this very exciting paper, Kerstin, Klaus and Heike Peterziel showed that although there is no upregulation of GDNF receptors mRNA and protein, TGFβ stimulates the movement of GDNF co-receptor GFRα1 from the cell to the plasma membrane. They provided additional evidence for their finding showing that the addition of soluble GFRα1 together with GDNF functions in the absence of TGFβ (Peterziel et al. 2002).

The discovery of GDF15 is another remarkable achievement in Klaus Unsicker's growth factor research (Böttner et al. 1999a, b). Although GDF15 is known as the key regulator or appetite and metabolism, Klaus initiated a completely novel direction in GDF15 research and focused on the neurotrophic effects of GDF15. Very soon after the discovery of the factor in several laboratories at the same time, he found that GDF15 is a novel neurotrophic factor with clear effects of dopamine and serotonergic neurons in vitro and in vivo. Importantly, they also discovered that GDF15 can protect and repair dopamine neurons in neurotoxin models of Parkinson's disease (Strelau et al. 2000). Current data indicate that GDF15 activates GFRAL-RET signalling solely in the hindbrain resulting in reduced food intake and loss of body weight. This indicates that GDF15 receptor GFRAL is expressed only in the neurons of the hindbrain region area postrema and not in other areas. Results obtained by Klaus and his team contradict this opinion. To analyse the role of endogenous GDF15 in the nervous system, Klaus and his colleagues developed and analysed GDF15 knockout mice. They found that GDF15-deficient mice exhibit progressive postnatal losses of spinal, facial and trigeminal motoneurons. What is more, sensory neurons in dorsal root ganglia were reduced by 20%, whereas sympathetic neurons were not affected (Strelau et al. 2009). These results are in conflict with the notion that GDF15 receptor GFRAL is expressed solely in the hindbrain neurons. Klaus and his team's results strongly suggest that motoneurons and also sensory neurons that are expressing either GDF15 receptor GFRAL or GDF15 can bind to and signal via an alternative, still unknown receptor. This is a burning unanswered question especially because pharmaceutical industry laboratories demonstrate considerable interest to GDF15 signalling pathway. Although GDF15 may have therapeutic potential for the treatment of several diseases, two main directions for the drug development for this central appetite-regulating protein can be delineated. Firstly, GDF15 or its analogues can be used as appetite suppressors to treat obesity. Secondly, blocking GDF15 signalling by using function-blocking therapeutic antibodies to GDF15 or to GFRAL or using RET antagonists can be useful for the treatment of anorexia-cachexia syndrome. It is very clear that it is of utmost importance to clarify, whether GDF15 signals solely via GFRAL-RET pathways or there is another alternative receptor-mediated pathway?

In addition to the studies on growth factors, Klaus has very important scientific contributions also to other research areas. I must also note that another impressive part of this scientific work product is the training of a cadre of junior scientists, many of whom contribute to the field. I would particularly like to point out the importance of Klaus work as the editor in chief of this journal and as the director and spiritual father of the Heidelberg Interdisciplinary Neuroscience Center. I would also stress that as a colleague (sometimes as the competitor), Klaus has a reputation as a gentleman with outstanding integrity.

Together with many colleagues from the growth factor field, we thank Klaus for his outstanding contributions and congratulate him on his eightieth birthday.

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