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Modeling chemotherapy induced peripheral neuropathy (CIPN) in vitro: Prospects and limitations

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Abstract

Neuronal cell cultures have been used as an essential tool for studying pathomechanisms of toxicity of chemotherapeutic drugs and to develop neuroprotective approaches. They offer the opportunity to dissect disease mechanisms and molecular pathways while allowing precise control of a variety of confounding factors of the physio-chemical environment. As such, a growing number of in vitro studies are published each year to decipher mechanisms of neurotoxicity of taxanes, vinca alcaloids, proteasome inhibitors and platin derivatives and/or to test neuroprotective strategies. Here, we provide a review of cell culture techniques and outcome measures that have been used in the past or are currently employed to model chemotherapy induced neuropathy in vitro. Furthermore, we discuss their advantages as well as their limitations and ways to enhance efficiency and reproducibility of cell culture studies in the field of toxic neuropathy.

Keywords

CIPN; Stem cell; Neurotoxicology; Regeneration; Cell culture technique; Cell culture conditions; Viability; Dorsal root ganglion neuron; Neuronal cell culture

1. Introduction

Peripheral neuropathy due to chemotherapeutic agents (CIPN) represents a particular challenge in the management of cancer patients. CIPN was first described in the late 1960s in patients receiving vincristine (Gottschalk et al., 1968; Moress et al., 1967) and since then has been recognized as the major dose-limiting side effect of many chemotherapeutic agents. Despite advancements in the development of novel treatment approaches such as immune checkpoint inhibitors (Assi et al., 2018; Ok and Young, 2017), antibodies against vascular-endothelial growth receptors (Shitara, 2017) and small molecules against intracellular targets, neurotoxic agents such as taxanes, platin-derivatives, vincristine and bortezomib will still be backbones of chemotherapy for solid cancer and hematological malignancies in the foreseeable future. CIPN is associated with reduced quality of life, due

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to pain, and increased risk of falls (Ewertz et al., 2015; Kolb et al., 2016). So far, no specific agents have been shown to be able to prevent CIPN and as such, the American Society of Clinical Oncology has recommended no agent for the prevention of CIPN.

Cell cultures are one of the most basic tools to study cellular and molecular effects of conditional changes in the immediate environment including exposure to potentially toxic compounds. Therefore, cell cultures are also frequently employed to model toxic effects of cytostatic compounds to cells of the peripheral nervous system (PNS). They allow controlling the physio-chemical environment such as pH, temperature, exposure to light and supply of nutrients. Moreover, they allow fast replication of an experiment under the same conditions and allow one to evaluate reproducibility. One of the major disadvantages of cell cultures in terms of translation, however, is that they are considered artificial. Usually, as a deconstructed model, only cells of interest are studied thereby neglecting the complex interaction of different cell types. For example, for CIPN research, dorsal root ganglion (DRG) neurons that are cultured in the presence of nerve growth factor (NGF), are often used as primary cell culture, and this cell culture condition selects NGF-responsive small diameter neurons. However, mammalian DRG contain many neuron subpopulations including small and large-diameter neurons in addition to perineuronal satellite cells, Schwann cells, resident macrophages and fibroblasts. Unless a co-culture system is used, cell culture using just neurons do not recapitulate the in vivo interactions among these cell types. In addition, cell culture does not adequately model pharmacokinetics of organ exposure, specifically the entry, distribution and exit of compounds to cells of the PNS which are - like the central nervous system (CNS) - protected by a blood-nerve-barrier (BNB). Finally, pure neuronal cell culture models fall short in modeling the effects of neurotoxic compounds that may be metabolically activated in the liver (Harry and Tiffany-Castiglioni, 2005). This is not relevant for the “traditional” neurotoxic chemotherapy agents but should be taken into account when novel drugs are examined.

Thus, effects that are observed in vitro are usually considered to be preliminary and require validation in a living organism. In such experiments, mostly rodents are used, which on the other hand may not adequately replicate the situation in humans. This dilemma favors the use of in vitro systems with human cells, although they are less frequently used compared to murine and rat neuronal cell cultures. Compared to animal models, cell cultures are more cost-effective and can be used as drug screening platforms. A further aspect that emphasizes the utility of cell culture models is the ethical dilemma of animal experimentation that is encountered by the adherence to the 3R principle (replacement, reduction and refinement). An overview of advantages and disadvantages of cell culture models and animal models are provided in Table 1.

In this review we will discuss cell culture models that are commonly used to study neurotoxic effects of chemotherapeutic agents with a focus on in vitro studies that use chemotherapeutic drugs vincristine, paclitaxel, cisplatin and bortezomib.

2. Cell culture models: Pros and cons

A literature search based on the before mentioned substances in conjunction with keywords “in vitro”, “cell culture”, and “neuropathy” revealed a total of 116 studies that evaluated pathomechanisms or preventive approaches in cell culture systems (Table 2).

Analysis of the studies revealed that in the majority of studies, primary cell cultures derived from rodents were used, mostly rat DRG neurons. Murine neuronal cells were also employed, but to a much lesser extent (Fig. 1). Twenty five percent of the analyzed studies used neuronal cell lines, most often PC12 cells. Human cell lines that were used include SH-SY5Y neuroblastoma cell line (Bavari et al., 2016) and more recently human iPSC derived neurons (Morrison et al., 2016; Wheeler et al., 2015; Wing et al., 2017).

The frequent use of primary cell cultures, derived from DRG neurons requires a closer look at the methodology of this cell culture system, its advantages and its limitations. Generally, DRG as a source of neurons for in vitro experiments has a long history (Bunge et al., 1980). Usually DRGs from embryonic or early postnatal rat pups are prepared by surgical preparation and are used as whole DRG explants or are subsequently dissociated by use of trypsin and/or collagenase. Even slight deviation from commonly used cell preparation protocols may result in different functional properties of cell cultures. For instance, trypsination followed by gentle mechanical dissociation has been shown to alter size and changes of endogenous potassium currents in HEK293 cells (Ponce et al., 2018).

In most studies DRG neurons are cultured in the presence of NGF, with concentration ranging from 3 ng/ml up to 100 ng/ml (Bobylev et al., 2016; Hol et al., 1994; Podratz et al., 2011). One has to bear in mind, that the surgical removal and dissociation of DRG already induces an experimental bias by “artificial” axotomy that activates similar signaling pathways seen after chronic constriction injury leading to hyperexcitability of dissociated neurons (Zheng et al., 2007) and the process of DRG dissociation itself upregulates TrkA, and TrKC receptors (demonstrated for trigeminal ganglia) (Genç et al., 2005).

Age of neurons represents an important confounding parameter for cell culture experiments (Ng and Lozano, 1999). For example, postnatal neurons rapidly downregulate TrkA receptor, which is required for NGF signaling (Bennett et al., 1996; Molliver and Snider, 1997), and may respond dierently to molecules of the environment, as demonstrated for myelin associated glycoprotein (MAG), which inhibits neurite outgrowth in postnatal, but promotes axon elongation in embryonic neurons (Filbin, 1995; Mukhopadhyay et al., 1994). Also postnatally, sensory neurons still mature with changing expression of signaling molecules and transcription factors, for instance in nociceptive neurons for CaMKIIα or TRPV1 (Isensee et al., 2017) and in mechanoreceptive neurons Runt related transcription factors (Runx) 1 and 3 (Yoshikawa et al., 2013). Thus, DRG neurons derived from adult animals are probably more appropriate for modeling CIPN, which primarily affects adults whose sensory neurons are fully matured.

Cell culture studies that are based on animal-derived tissue always raise the question to what extent these results can be extrapolated to the situation in humans. Recently, Schwaid and colleagues compared the proteome of rat and human dorsal root ganglia and found that

the DRG proteome is largely (> 75%) congruent which supports the concept of principal translatability, within the constraints that potential differences in protein quantity, function and dynamic changes are not taken into account (Schwaid et al., 2018)(Fig. 2). Species differences have been reported regarding the response to cisplatin and bortezomib in DRG neuronal cell cultures derived from either rat or mice, to the extent that mouse DRG neurons are more resistant to toxic effects (Podratz et al., 2016).

Moreover, also among mouse strains, the sensitivity to toxic effects varies; for example, cisplatin is more toxic to neurons derived from C57BL/6J mice compared to those stemming from C3H/HeJ mice (Podratz et al., 2016). Despite the above shortcomings, the use of sensory neurons in vitro allows dissecting pathomechanisms at a molecular level, which explains its attractiveness as cell culture system to model CIPN. Compared to a cell line, such as PC12 cells, it may replicate more accurately the situation in vivo, since DRG neurons represent the cell type that is targeted by chemotherapeutics. Furthermore, conducting mechanistic experiments with DRG neurons in vitro is more efficient in terms of time and costs compared to in vivo animal experiments. However, the use of NGF as growth factor selects only NGF responsive (p75 and TrkA positive) neurons, which correspond to small, un- or only thinly myelinated nerve fibers in vivo. It has to be considered that DRGs of higher vertebrates comprise > 20 different subtypes of sensory neurons (Friedel et al., 1997). Moreover, there is abundant clinical and autopsy evidence that the neuropathy caused by paclitaxel, cisplatin and bortezomib affects also, if not predominantly, large sensory nerve fibers (Chaudhry et al., 2008; Krarup-Hansen et al., 1993; Krarup-Hansen et al., 2007; Sahenk et al., 1994). A way to bypass this selectivity of explored neuronal subpopulation and to more closely model the disease condition, one could use neurotrophins such neurotrophin 3 (NT3), glial derived neurotrophic factor (GDNF) (Gavazzi et al., 1999) and brain-derived neurotrophic factor (BDNF) that support other sensory neuronal lineages.

DRG explants and even dissociated neuronal cell cultures contain almost invariably to some degree non-neuronal cells such as Schwann cells and fibroblasts. Depending on the desired cell culture condition, depletion of these cells can be achieved by mechanical approaches (Jirsova et al., 1997) or with mitotic inhibitors such as Floxuridine (FUdR) (Malin et al., 2007) or cytosine arabinoside (AraC) (Wood, 1976), but these agents may be toxic to neurons as well (Wallace and Johnson Jr., 1989; Zhuo et al., 2018) and thus confound experimental outcomes. Neuronal cell cultures that are basically free from non-neuronal cells may be better monitorable, (i.e. measuring direct response to neurotoxic drugs), and may be analyzed by standard methods like Western-Blot and ELISA to monitor intracellular signaling cascades. However in the nervous system of vertebrates, virtually all peripheral axons are engulfed by Schwann cells, even without assembling myelin, and also DRG contain, apart from neurons, a myriad of non-neuronal cells including macrophages, fibroblasts, satellite glial cells and Schwann cells. Therefore, mixed co-cultures must be considered less artificial compared to pure neuronal cell cultures. Mixed cultures, on the other hand, may require advanced single cell analysis techniques in addition to immunostaining, such as single cell RT-PCR (Ho and O'Leary, 2011) or a quantitative automated microscopy (Andres et al., 2010), when signaling cascades and response to stimuli are investigated. Markers that are often used to label specific cell populations are β III tubulin for neurons and S100 for Schwann cells. Same limitations must be considered

when pure Schwann cell cultures, in the absence of any neurons, are used (Campana et al., 1998; Imai et al., 2017).

3. Cell lines to model CIPN

The most frequently used cell lines to model CIPN in vitro are PC12 cells, (SH)-SY5Y cells, and immortalized DRG neurons. PC12 cells originate from rat phaeochromocytoma, divide and secrete catecholamines (mostly dopamine and noradrenaline). When exposed to NGF, PC12 cells stop dividing and develop to a neuronal phenotype that extend neurites, become electrically excitable, and establish synapses when co-cultured with muscle cells (Fujita et al., 1989). Although being a cell line, variability exists among PC12 clones in terms of protein expression and extension of neurites (Clementi et al., 1992; Koike et al., 2017). High number of passages alters the sensitivity to toxic compounds and may lead to misinterpretation of toxic or neuroprotective interventions (Kinarivala et al., 2017). Despite these concerns, studies that used PC12 reported overall consistent effects; for example for cisplatin (in a dose of 32 μM), a reduction of undifferentiated PC12 cell viability ranging from 40 to 50% over 24 h can be expected (Li et al., 2015; Li et al., 2019; Mendonca et al., 2009).

SH-SY5Y is a human neuroblastoma cell line and can be differentiated by retinoic acid, dibutyryl cyclic AMP (dbcAMP), or neurotrophins into mature human neurons that express neurites (Kovalevich and Langford, 2013). Due to expression of tyrosine hydroxylase and acetylcholine receptors they are frequently used as a model for dopaminergic or cholinergic, but a less common model for sensory neurons. Nevertheless, a comparison of the transcriptome between SH-SY5Y and murine DRG revealed, that SH-SY5Y express many markers that are present in peripheral sensory neurons such as RET, GDNF receptor tyrosine kinase, and TrkA, although the expression profile is not characteristic for a specific subclass of peripheral sensory neurons (Yin et al., 2016). Importantly, they lack the nociceptive neuron marker, transient receptor potential vanilloid family-1 (TRPV1), and the peptidergic neuron marker CGRP, which makes this cell line not suitable for drug screening approaches related to CIPN associated neuropathic pain. A clear advancement in this regard is the establishment of an immortalized rat DRG neuronal line, 50B11 (Chen et al., 2007). These cells can also be differentiated into a neurite-extending phenotype, and express markers such as p75, TrkA, c-ret and GFRa1. After exposure to cisplatin or paclitaxel, these cells show a reduction in neurite length (Vencappa et al., 2015; Zhu et al., 2013), and mitochondrial dysfunction (Galley et al., 2017).

4. Human cell-based models

4.1. hESC-derived neuronal cells

Despite substantial similarity in the protein expression of human and rat sensory neurons, the use of human neurons is preferred and considered less artificial. The stem cell technology offers the opportunity to culture human neurons. One approach is the use of human embryonic stem cells (ESCs) that are totipotent and induced into neurons by use of specific cell culture conditions (Jones et al., 2018). It has been demonstrated that sensory neurons derived from hESC are heterogeneous with regard to expression of specific markers

for neuronal subpopulation and as such comparable to the situation in vivo (Alshawaf et al., 2018).

4.2. hiPSC-derived neuronal cells

Human induced pluripotent stem cells (hiPSCs) offer an alternative to generate sensory neurons and have already been employed by several research groups to study neurotoxicity (Hoelting et al., 2016; Wheeler et al., 2015; Wing et al., 2017). Interestingly, neurons derived from genetically different hiPSCs also displayed differences in terms of neurite outgrowth in the presence of paclitaxel which emphasizes its suitability as a genetically diverse human cellular model for CIPN (Wheeler et al., 2015). Rana and colleagues recently demonstrated the use of hiPSCs as tool for high throughput screening (Rana et al., 2017). Limitations of hiPSC include tedious and time intensive cull culture conditions with low efficiency in terms of reprogramming and genetic instability. Direct conversion of somatic cells (i.e. fibroblasts) to functional neurons may offer an alternative to circumvent these hurdles (Hoelting et al., 2016).

5. In vitro outcome measures and their relevance to human disease

Just as there are major differences in the use of cell culture systems and techniques, there are also major differences regarding the use of in vitro outcome measures. These include mainly measures of cell viability, axon morphology and biochemical assays. Although all these measures have their advantages in terms of feasibility, robustness, and reproducibility, these returns do not necessarily correspond to relevance and usefulness for further translation to in vivo models or to the human condition CIPN. In humans, CIPN is a predominantly sensory axonal neuropathy that is neuropathologically characterized by a “dying back” axon degeneration that proceeds in a distal-to-proximal fashion. An exception to this is cisplatin induced neuropathy, which causes a sensory neuronopathy, with neuronal cell death at the level of the DRG (Staff, N.P, et al., 2017). Therefore, an assay that measures axonal degeneration might be more suitable to address paclitaxel induced neuropathy, whereas a “live-dead” assay that measures cell viability might be more translatable to cisplatin induced neuropathy. In the following sections we will discuss the most frequently employed assays.

5.1. Cell viability

Viability of neuronal cell lines or non-neuronal cells is a frequently used endpoint in in vitro studies. A conceptual limitation of cell viability assays is that other mechanisms than just neuronal cell death may contribute to the neurotoxic effects in vivo and functional impairment may occur even at sublethal concentrations (Harry and Tiffany Castiglioni, 2005). Moreover, cell populations studied in vitro are usually not in the same stage of their cell cycle. Thus they may respond differently to cytostatics with some cells undergoing cell death while other (non-dividing) cells may survive. This is particular important for primary neuronal cell cultures that use (immature) embryonal DRG neurons but heterogeneous cell cycle may also bias cytotoxicity experiments in neuronal cell lines. To bypass this phenomenon, experimental paradigms with varying (i.e. longer) incubation times and multiple time points of measurements are recommended (Ramirez et al., 2010).

Cell viability can be assessed with fluorescent probes such as propidium iodide (to label dead cells) (Ustun et al., 2018), fluorescein diacetate (FDA, producing green fluorescence in living cells) (Aubert et al., 2008), colorimetric WST-8 assay (Kawashiri et al., 2018), or TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining (Melli et al., 2008). A drawback of assays that are based on dyes that enters the compromised membranes of dying cells is that a proportion of these cells may still retain their membrane integrity for a substantial period of time after injury (Ramirez et al., 2010). In CIPN models with primary cell lines, a common problem is to identify apoptotic neurons in mixed cultures from non-neuronal cells, which requires an additional staining and washing procedure which may detach dead cells and hence introduce bias. These markers could be NeuN, which is expressed exclusively in neuronal cell nuclei or Tuj-1 which stains neuronal cell bodies and neurites. Vimentin is a specific biomarker, compared to S100 and GFAP, to stain cell bodies and proprocesses of non-neuronal cells according to a study by Guo and colleagues, (Guo et al., 2017).

Viability assays that are based on assessment of metabolic function are the MTT assay and quantification of ATP. The MTT assay is based on enzymatic conversion of a tetrazolium compound to insoluble formazan crystals, indicating living cells (van Tonder et al., 2015). Other modified tetrazolium-based assays are the MTS and WST assays. The MTT assay is considered gold standard for cytotoxicity assessment, but might be biased by mitochondrial number and function that influence the conversion to formazan crystals (van Tonder et al., 2015), medium conditions including serum and albumin, and growth state of cells (confluent or exponential) (Liu and Dalgleish, 2009; Stepanenko and Dmitrenko, 2015). Notably, Ulukaya and colleagues reported that cisplatin and paclitaxel can increase absorbance values of the MTT assay, which may lead to false positive overestimation of cell viability (Ulukaya et al., 2004). A way to improve accurateness of cell viability, combination of assays might be considered either by trypan blue staining or by adding conventional microscopic assay (Garg et al., 2018). Cell death goes along with rapid depletion of ATP, which is exploited by luciferase-based ATP detection assays. However, different cell types may have different amounts of ATP and cell culture conditions may further influence ATP content by contact inhibition at high densities. A major advantage of cell viability assays is that they are easily adaptable for high throughput screening and may be used in an initial screen for potential neuroprotective compounds using robotic systems (Schmidt et al., 2017).

5.2. Morphological measures

5.2.1. Neurite length—Measurement of neurite length is one of the most commonly used assays to investigate axon degeneration in vitro. Usually dissociated cell cultures are stained with a neuronal marker (β III tubulin, neurofilament) and length of neurites, either total or longest, is measured by use of an image analyzing program, optionally facilitated by semi-automatic imaging processing programs (Long et al., 2017). Furthermore, several automated image processing algorithms allow one to develop high content screening assays to examine potential neuroprotective compounds (Chen et al., 2015; Rudhard et al., 2015). In case DRG explants are used, radial neurite length is assessed. Depending on how this assay is structured it can measure actual axon degeneration (e.g. by allowing the axons to extend for a period of time before adding the toxic and/or protective compounds) or

inhibition of further neurite outgrowth (i.e. secondary regeneration after the initial injury induced by culturing of DRG neurons). Despite this conceptual shortcoming, the neurite length is often considered to be an assessment tool for axon injury in general. In studies that used microtubule stabilizers, also the branching of axons is assessed by counting the number of branching segments per a given field (Pittman et al., 2016).

5.2.2. Neurofilament—Axons contain intermediate filaments composed of neurofilament (NF) subunits, of different size, namely, NF-L (68 kDa), NF-M (150 kDa) and NF-H (190–210 kDa)(Hares et al., 2011). Phosphorylation of NF is a marker of axon integrity and dot blot assay has been proposed as an in vitro assessment (Hares et al., 2011). In vivo assessment of NF-L in body fluids has emerged as novel biomarker for axonal damage in many neuropathic conditions, including CIPN (Mariotto et al., 2018; Meregalli et al., 2018). In vitro studies have used staining for NF-L as marker for axonal loss (Jackson et al., 2018), but to date it is unknown if an immunoassay for NF-L in cell supernatants may also serve as a marker to assess axon integrity in neuronal cell cultures.

5.3. Electrophysiological recordings

Whole and dissociated DRGs can be used to perform electrophysiological recordings by use of patch clamp technique. It allows studying channel and receptor function of neurons in vitro. Conceptually, it is anticipated that ion channels expressed in the membrane of the cell soma are congruent to those of nerve terminals which are not accessible to microelectrodes (Passmore, 2005). Basically, a glass electrode is sealed onto a membrane patch to measure rapid ion channel-mediated conductance changes across a neuronal membrane (Hoerbelt and Heifets, 2018). Likewise, intracellular activity can be recorded by use of appropriate sharp glass electrodes.

By use of these techniques, it could be demonstrated that paclitaxel evokes ectopic spontaneous activity in neurons in vitro which is caused an increased expression of the ion channel $Na_v1.7$ (Li et al., 2018; Zhang and Dougherty, 2014). Another example includes oxaliplatin, which alters currents of voltage-gated calcium channels (Leo et al., 2017; Schmitt et al., 2018). An extension to these single cell recordings is the use of multi-well multielectrode arrays to record spontaneous activity in whole DRG cell cultures (Newberry et al., 2016).

6. Summary and future directions

Over several decades cell cultures have served as a valuable tool to study neurotoxicity with a growing diversity in terms of used cell lineages, outcome measures and molecular/biochemical methods. As much as this diversity is to be welcomed, it makes it more difficult to compare observations and to assess its overall significance. Some form of standardization in the field is necessary. For example, approaches to align in vitro testing for CNS toxicity has already been proposed and validated around 30 years ago (Atterwill et al., 1993; Atterwill and Walum, 1989; Williams et al., 1994). This approach employed a three tiered in vitro testing procedure for neurotoxicity with screening experiments based on human and neuroblastoma cell lines and rat primary mixed neural cell cultures with endpoints including MTT reduction and LDH release (first tier). As second tier organotypic cultures

were proposed for investigation of kinetics, specificity and patho-mechanisms. A third phase included experiments using specific neural and astrocytic cell cultures. A potential modification for the purpose to study CIPN in vitro is shown in Fig. 3. However, such general recommendations and guidelines for conducting experiments are only useful if they are implemented over the long term, and widely accepted in the research community (the above mentioned papers were last referenced more than ten years ago). Furthermore such recommendations would need to be quickly adapted and regularly updated in line with the latest methodological and scientific developments. Some of these future developments might be the more extensive application of stem cell technologies or novel cell culture techniques such as 3D cell cultures. Particularly the latter may help to improve translation of findings to the next pre-clinical level in small animals. Examples include the use of gelatin blocks with capillary structure to model the 3D structure of nerves (Anderson et al., 2018) or spheroids consisting of Schwann cells and neurons (Kraus et al., 2015).

On a conceptual level, more rapid dissemination of research findings and closer collaboration with exchange of knowledge in methodology of research groups in the field of neuroscience, but also oncology, may help to design future research in a more efficient way. As such, the recently founded Toxic Neuropathy Consortium (TNC, https://sites.google.com/campus.unimib.it/tncwebsite/home-page) may represent a step forward to optimize pre-clinical research in the field of CIPN.

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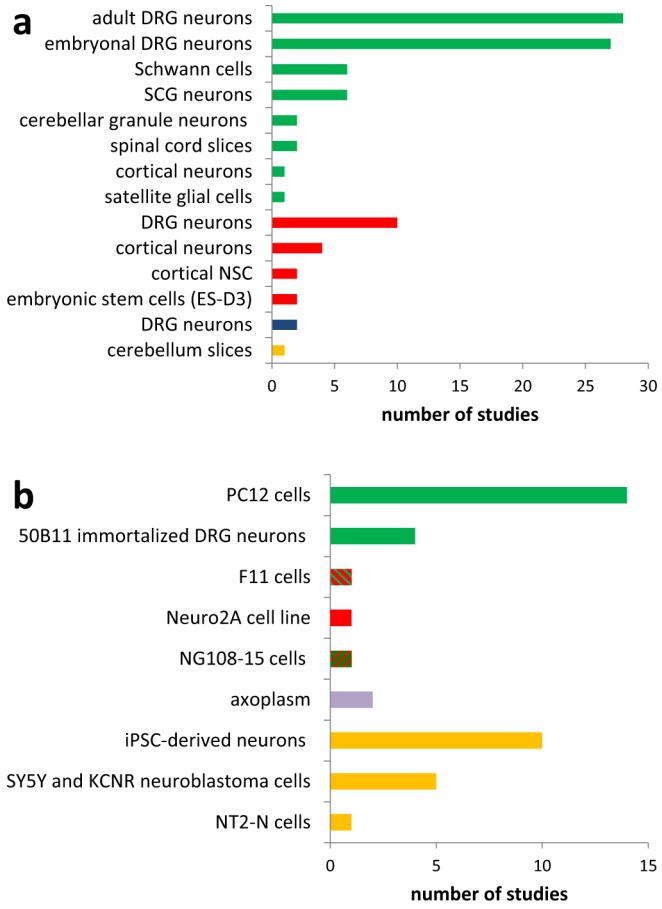

Fig. 1.

Overview about employed cell culture systems to model chemotherapy induced peripheral neuropathy. a: primary cell cultures, b: cell lines. Species are labeled with colors (green = rat, red = mouse, blue = chicken, purple = squid, yellow = primate/human).

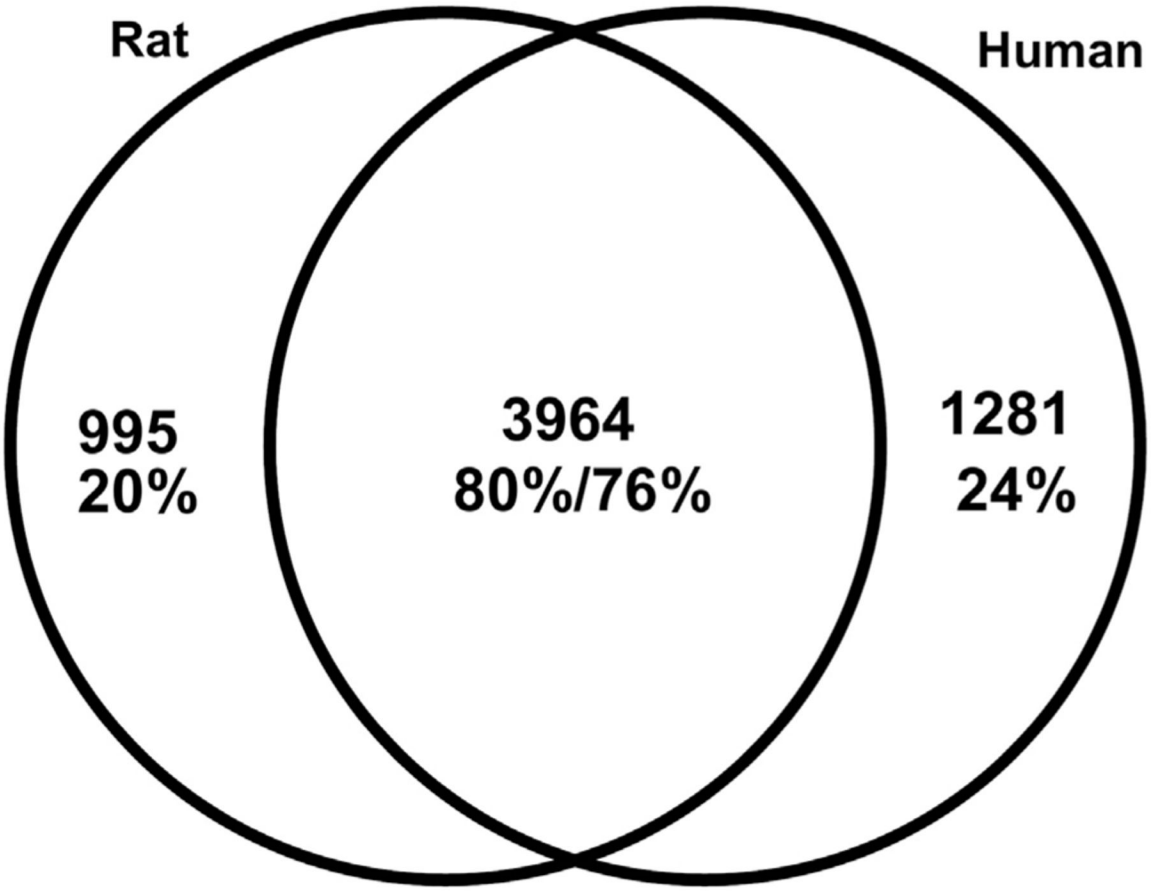

Fig. 2.

Venn-Diagram of common and unique expressed proteins in rat and human DRG proteome. There is a large overlap in the proteome of both species (reprinted with permission from (Schwaid et al., 2018)).

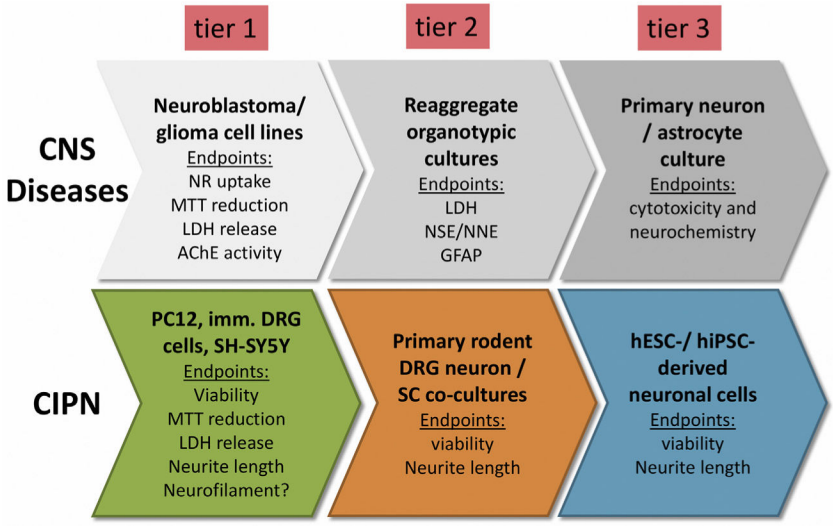

Fig. 3.

Three-tiered-test model to study cytotoxicity. The upper sequence shows a three-tiered test model to study CNS cytotoxicity (modified after (Atterwill et al., 1993; Williams et al., 1994), the lower line a potential modification to study CIPN in vitro. NNE = non-neuronal enolase. NSE = neuron specific enolase.

Table 1

Pros (in green) and Cons (in red) for different experimental approaches.

Cell culture approach	Animal experimentation
fast	time consuming
separate effects can be better studied (deconstructed approach)	complex interactions / microenvironment less well controlled
simplistic approach, only cells of interest are studied	less artificial due to interaction of different functional systems /organs / cell types
control of potentially confounding factors	potentially confounding factors are less well controlled
cheap	expensive
does not reflect potential metabolism of compounds to active metabolites in non-neuronal tissues	More suitable for PK/PD studies
	ethical dilemma

Table 2

Search strategy for reviewed papers. Causes of exclusion were other article types such as review, clinical trials etc., or research unrelated to CIPN.

Keywords used	n	Included
Cisplatin AND neuropath* AND (in vitro OR cell culture)	88	38
Cisplatin AND neuropath* AND stem cell	38	4
bortezomib AND neuropath* AND (in vitro OR cell culture)	33	10
Bortezomib AND neuropath* AND stem cell	153	2
Vincristine AND neuropath* AND (in vitro OR cell culture)	39	13
vincristine AND neuropath* AND stem cell	43	3
Paclitaxel AND neuropath* AND (in vitro OR cell culture)	129	43
Paclitaxel AND neuropath* AND Stem cell	33	3