



Research Paper

Expression of the *pokemon* gene and pikachurin protein in the pokémon pikachu

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ABSTRACT

The proto-oncogene *Pokemon* is typically over expressed in cancers, and the protein Pikachurin is associated with ribbon synapses in the retina. Studying the former is of interest in molecular oncology and the latter in the neurodevelopment of vision. We quantified the expression levels of *Pokemon* and Pikachurin in the Pokémon Pikachu, where the gene and protein both act as in other vertebrates. The controversy over their naming remains an issue.

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INTRODUCTION

Pokemon is a proto-oncogene discovered in 2005 (Maeda et al., 2005). It is a “master gene” for cancer: over expression of *Pokemon* is positively associated with multiple different forms of cancer, and some hypothesize that its expression is a prerequisite for subsequent oncogenes [cancer-causing genes] to actually cause cancer (Gupta et al., 2020). The name stands for POK erythroid myeloid ontogenic factor. [POK stands for Pox virus and Zinc finger domain, Krüppel-like]. The contemporary, more politically correct name for this gene is *Zbtb7*, which stands for Zinc finger and BTB domain-containing protein 7A, with BTB standing for “*BR-C*, *ttk*, and *bab*,” which respectively stand for the fruit fly genes *Broad-Complex*, *tramtrack*, and *bric à brac* (Zollman et al., 1994). Other names include LRF10 (leukemia/lymphoma-related factor), OCZF11 (osteoclast-derived zinc finger), and FBI1 (fourteen-three-three beta interactant) (Choi et al., 2008). We will call it *Pokemon* from here on out. Pikachurin is a lighting-fast retinal protein discovered in mouse eyes involved in normal photoreceptor ribbon synapse formation and normal visual perception (Sato et al., 2008). It is also known as AGRINL, short for agrin-like, referencing a protein involved in the development of neuromuscular synapses in embryos (Campanelli et al., 1992). It is also known as EGFLAM, short for Epidermal growth factor-like, fibronectin type-III and laminin G-like domain-containing protein, and is thus coded by the gene *EGFLAM*. It is active within the extracellular matrix between the photoreceptor and bipolar cell neurons of the retina, forming a “ribbon synapse” capable of extremely fast neurotransmission. While the above abbreviations can be

confusing, they do avoid the controversies associated with naming a disease-related gene after adorable, child-friendly creatures. [For more information, consider the holoprosencephaly-associated gene sonic hedgehog and the molecule that inhibits it, Robotnikin (Stanton et al., 2009)].

The gene *Pokemon* is thus not to be confused with Pokémon, short for pocket monsters (*Sinum Monstrum*), a clade of organisms initially described by Tajiri and Miyamoto (1996) and Tobin et al. (2004), with 890 species presently described by Shelomi (2019). The protein Pikachurin is not to be confused with the individual Pokémon named Pikachu (Figure 1). Pikachu (*Electricamus scintilla fulgur*) is one of several closely related electric rodents formerly synonymized under *Mus electrica* (Shelomi et al., 2012). Pikachu are known for generating electricity in electrocytic organs and storing it in their cheek pouches (Stoll, 2017), their correlation with anomalous lightning storms and their propensity to wear hats (Spiegel et al., 1998). Given the importance of speedy processing of vision to a creature noted for its quick attacks and lightning-fast reflexes, combined with the desire for Pikachu trainers that their friends not die of cancer, we used qPCR to compare expression levels of the Pokémon gene and the Pikachurin protein across multiple wild-caught and laboratory-trained samples of the Pokémon Pikachu. The goal is to understand the link between proto-oncogenes and tumor genesis in the electric mouse, test the hypothesized presence of ribbon synapses in electricocytes and demonstrate how the absence of peer review in predatory journals can lead to the blurring of the lines

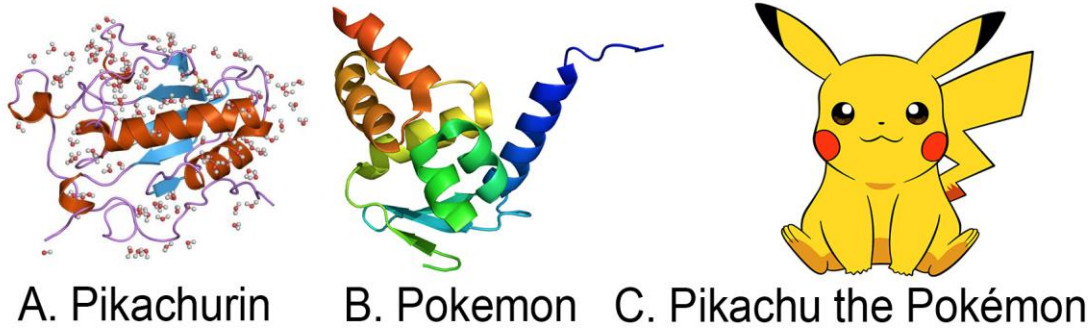


Figure 1: Pikachu

between science and fiction in ways that crypto zoologists have only dreamed of.

METHODS

As per the ethical research guidelines of the Institutional Committee for Utilization of Pokémon (ICUP), any Pokémon transferred to a Pokémon institute cannot be used for research purposes unless the transferring trainer previously signed a consent form designating their transfers for such usage. Thus, wild caught Pokémon were necessary. After appropriate permits were obtained from the Kanto Department of Environmental Management and the National Tall Grass and Safari Zone Conservation Departments, wild Pikachus were caught by trainers throughout the Kanto region and transferred to the Okido Institute via Pokémon Storage System (Bill's PSS v.4.20). In addition, local PokéPest exterminators were asked to transfer all wild Pikachus caught damaging grass with their electricity or damaging electronic equipment to the Okido Institute. We also took non-destructive samples from trained and laboratory-reared Pikachus brought to the institute by Pikachu trainers and breeders volunteering to assist in the study. After a five day recovery and acclimation period in natural conditions with *ad libitum* access to fresh water and berries, Pikachus were anaesthetized with aerosolized Morellul® Sleep Powder. Age and vital statistics were recorded, and a health-scan performed under the auspices of a registered Pokémon Nurse and research-certified Chansey. Using electrically-insulated PPE and operating within a lightning-proofed facility, a full-thickness retinal biopsy (Hellsing et al., 2012) of the left eye and a subcutaneous electrocyte biopsy (Burns and Smithers, 2014) of the left cheek pouch and the tail tips were performed on each Pikachu, as these tissues are hypothesized to express Pikachurin. A biopsy ear punch was also performed to produce control tissue. All procedures were approved by the Pokémon Care and Use Committee of Kanto. Following the procedures, Pikachus were brought back to full health at the Viridian City Pokémon Center, as confirmed with a standard vision test

and bilateral thunderbolt generation assay (Sanchez and Smith, 2019). These were then released to the wild, returned to their trainers, or converted into Pichu candy as appropriate. We used the minimal sample size needed to produce statistically meaningful research, and conformed to the ARON (Association for Research in Ophthalmology and Neurology) statement for the ethical use of Pokémon in medical research.

If any tumors were detected in the scan, they were biopsied as appropriate for the tumor type and used for detection of the *Pokemon* gene, along with a biopsy from tumor-free tissue as a negative control. After recovery, such Pikachu were transferred to the Viridian City Pokémon Center Oncology Department for further evaluation and treatment if appropriate. In addition, frozen Pikachu cancer tissue from the Viridian City Veterinary Oncology tissue bank was sampled if it had been designated as available for research purposes (Man et al., 2007). To quantify expression of *Pokemon* and *EGFLAM*, the latter used as an indicator of Pikachurin protein expression, we used qPCR. Tissue samples were homogenized in TriZol solution with a beat beater and RNA extracted with a GENesect Kit. RNA was kept frozen at -80°C until needed. qPCR was performed using published primer pairs for *Pokemon* (Hatt and Conductor, (2007); Montague et al., 1597), *EGFLAM* (Frankenstein et al., 2005), acting as positive control, and GFP as a negative control. The PCR conditions are as in those citations, and any further methods are unnecessary as this is a predatory journal (Ramsay et al., 2018).

RESULTS

Though over 150 were collected, we only performed retinal and electrocyte biopsies and *EGFLAM* qPCR on 60 Pikachu (30 male, 30 female). High expression of *EGFLAM* was detected in the retinal tissues of all individuals, except one that was subsequently diagnosed with retinal muscular dystrophy. It was cured with a hyper potion. No *EGFLAM* expression was detected in control or electrocyte tissues. We obtained fresh tumor tissue samples from 20 Pikachu (6 skin, 4 colorectal, 4 electrocyte, 3 lung, 2 bone, 1 ovarial).

Most were premalignant, except for 2 benign skin tumors and one malignant lung tumor. All but the benign tumors expressed high levels of *Pokemon*. All Pikachu were successfully treated. The Viridian center tissue samples have been subsequently analyzed more in depth and are fully described in another paper (Read et al., 2004), but we can state here that all except a brain gliomas and one pancreatic sarcoma expressed high levels of *Pokemon*.

DISCUSSION

Pikachurin expression was found as expected in retinal tissue, but not in electrocyte tissue. This finding is as expected with previous work on the transcriptomics of Pachirisu (*Electricamus sciurus*) that found no EGFLAM expression in the electrocytes (Blofeld et al., 1958). We thus confirm that the protein is expressed in Pikachu as in other vertebrates. Future research into Pikachurin can look at the expression of this protein in embryonic Pichu and its role in development, or identify EGFLAM mutations associated with visual, electrical and other nervous-system related disorders in the electric mice. Expression of *Pokémon* above our detection limits was only found in tumor tissue from Pikachu. While further research is needed on the natural function of the *Pokemon* gene in healthy Pokémon, its link with cancers has been noted in several previous studies, including laboratory Rattata models of human cancers. *Pokemon* over expression has previously been linked to fibrous osteosarcoma in Cubone (House et al., 2005), childhood astrocytoma in Abra (Lennon et al., 1960), metastatic melanoma in Solrock (Sparkle, et al., 2015) and pleuropulmonary blastoma in multiple pokémon species following prolonged cohabitation with Galarian Weezing (Pickles et al., 2009). Continued research into *Pokemon* in Pokémon could lead to great strides in Pokémon oncology and help devise treatments for conditions not treatable by potions or healing moves. Several points of contention exist surrounding these results. Some would argue that research into the transcriptomics and proteomics of Pikachu is unnecessary, given that it is a fictional creature.

We counter that Pikachu is as real and existent as this journal's peer review process (Masic, (2017), Roberts, (2016) and Vakil 2019). Many academics, particularly naïve scientists and/or those in developing nations, still publish papers in such fraudulent journals, spending considerable sums of limited funding for what are ultimately glorified blogs (Gopalakrishnan et al., (2016); Xia et al., (2015) and Tella 2020). While over-reliance on statistics such as impact factor or lists such as the Web of Science Master Journal List as its own drawbacks, academic institutes need to find some way of separating the wheat from the chaff. Namely: if a scientist publishes in a non-peer reviewed journal [such as literally any journal operated by OMICS and Longdom Publishing, which are one and the same], they should receive zero credit or recognition for it when their publication records are reviewed for purposes

of promotion or hiring or tenure. Institutions especially in the developing world, should also educate their researchers on the existence of predatory journals with the same rigor that they teach laboratory safety.

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