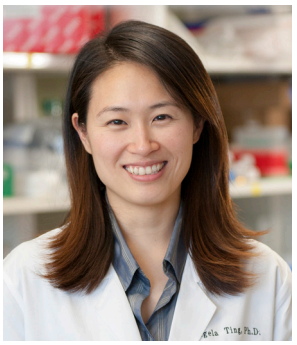
WOMEN IN CANCER PROFILE

Dude, where's my band?



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Halfway through my third year in college, I still had not decided on a career path. I toyed with the idea of going to law school to become an intellectual property lawyer. I considered undergoing further training to become a genetic counselor. I also attended several career opportunity seminars by notable consulting firms. None of these possibilities really excited me. Coincidentally, I had started attending a superb journal club led by Dr Carl Douglas at the University of British Columbia. By far, it was one of the most enjoyable classes I had taken because we were reading and discussing research studies that were pushing the frontiers of knowledge. The articles did not always provide all the answers to the question(s) the authors set out to address and often raised additional inquiries and fueled further curiosity. I thought to myself, 'what an adventure it must be to solve the mysteries of life for a living?'

This journal club not only sparked my interest in research but also ignited my curiosity about epigenetics, which are non-sequence altering modifications to chromatin that result in heritable changes in gene expression. The functional genome is the culmination of both the hard-coded DNA sequence and the dynamic epigenetic marks imposed upon it. Understanding aberrant epigenetic modifications in pathological conditions is vital for disease intervention. This spark set off a chain reaction consisting of taking my graduate record examinations (GREs), filling out graduate school applications and interviewing with potential thesis advisors.

I was fortunate to further develop my interest and technical abilities in epigenetics as a graduate student with Dr Stephen Baylin at the Johns Hopkins University School of Medicine. At the time of joining his lab, I indicated to Dr Baylin my desire to test if small RNA molecules could serve as initiators of epigenetic remodeling in human cells, similar to RNA-dependent DNA methylation in *Arabidopsis thaliana* (Wassenegger & Pelissier 1998). Although this was a risky project and was outside of his primary research focus, he gave me the freedom to explore. Like most graduate students, I encountered many stumbling blocks along the way, and at times, thought it was impossible to reach graduation. There were also occasions when I was desperate for results, any results, that some sort of data dance or bacterial culture sacrifice was seriously contemplated.

My most memorable roadblock happened in my first year in the lab. I was attempting to test my hypothesis by targeting the cadherin 1 (*CDH1*) promoter with synthetic small double-stranded RNAs to see if I could induce epigenetic remodeling and modify transcription activity. Before I could begin the actual experiment, I needed to make sure I could robustly detect both mRNA and protein expression in the cell lines I was using. I was a novice at mammalian cell culture and followed the instructions of a senior postdoc to a tee. I mastered sterile techniques and maintained healthy cells. I harvested glistening, pearl-sized pellets of cells and prepared high-quality RNAs and cell lysates suitable for mRNA expression and Western blot analyses. My RT-PCRs yielded correct products. My gels for Western blot ran straight and even, and my transfer was bubble-free. Then came the moment of truth in the dark room. My blot for CDH1 was blank! Not a single speckle

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or a shadow of a band! I re-probed the same blot for β-actin, and expecting a blank blot, I was shocked to see a blazing, unequivocal band at the correct molecular weight for β-actin.

Befuddled, I repeated the experiment several more times, and each time, I double and triple checked my steps and reagents. Yet each time, my blots for CDH1 were completely blank, and my re-probing for β-actin would be beautiful. Determined to figure out what was going on, I tested almost all the antibodies in the lab and even a few from our neighboring labs. Blot after blot after blot, I could detect everything but CDH1. I used different cell lysis buffers, I varied my blot transfer parameters, I tested out other types of gels, I altered my probing conditions, and I ordered anti-CDH1 antibodies from all available commercial sources. By the time Christmas rolled around and 6 months after I started in lab, I still could not see CDH1 on a Western. My frustration was reaching a tipping point. I was deflated. I was losing confidence. I was beginning to doubt the decision of going to graduate school.

One evening, I was sitting at my sad bench staring at my sad blank blots, it dawned on me. I had troubleshot every aspect of my protocol after the collection of cell pellets. 'Could I have done something wrong with my cells?' I wondered. I retraced my steps carefully in my head – seeding cells, checking cell confluency, rinsing cells with sterile PBS, adding trypsin, putting the flask back in the incubator for a few minutes to allow trypsin to chew up cell adhesion proteins so cells would detach from the flask, taking the flask out of the incubator, adding culture media to neutralize the trypsin, collecting cells into a conical tube, centrifuging the tube to collect the cell pellet, rinsing the cell pellet and storing the cell pellet in the –80° freezer. 'Wait a second!' I yelled at myself, 'Back up!' I muttered the sentence once more under my breath, '… putting the flask back in the incubator so trypsin can chew up cell adhesion proteins…'.

It has been 15 years since that instant of clarity. I cannot recall if tears of joy were flowing, but it was unmistakably my first eureka moment in my scientific career. It was not quite the pushing-the-frontiers-of-knowledge discovery I envisioned graduate school would bring, but it was a powerful revelation carrying a lasting impact on my attitude and approach to research. I could not wait to harvest my cells, this time, using a cell scraper. I could not wait to repeat my Western blot for CDH1 using this new cell lysate. I anxiously waited for the developer to spit out my film. Then, there it was – a pristine band at 120 kDa, the proof that the cells indeed expressed CDH1, and a lesson I needed to learn about research.

The lesson, which seems so obvious, is that critical thinking is vitally important to the scientific process. If I had analyzed each step of the cell culture protocol as opposed to simply following instructions, I might have asked earlier if there were multiple ways of harvesting cells. If I had noticed that all the antibodies I tested were against intracellular proteins except for CDH1, I might have realized sooner the problem with trypsin. Such critical evaluation is a necessity in performing experiments and interpreting experimental results. It took me nearly an entire year to come to this realization, but I hope it will serve me well for an entire career. I spent the next three years testing my hypothesis and eventually published my findings that showed that small double-stranded RNAs could potentiate epigenetic silencing in human cancer cells (Ting *et al.* 2005).

Of course, the elusive CDH1 band was neither the last hurdle to testing my hypothesis nor was it to completing a body of work worthy of a PhD thesis. Besides critical thinking, staying curious, having the courage to go off the beaten path, being creative and striving for consistency were a few more characters I picked up along the way. I practice these lessons daily, and they have served me well in establishing my own research program. My current adventure in epigenetics consists of two components aimed at making discoveries that, hopefully, will improve our ability to treat and prevent cancer. First, a pressing challenge in the clinical management of prostate cancer is to be able to accurately identify and extirpate aggressive tumors that will invariably result in patient death, while sparing men with indolent cancers the morbidity of radical treatment. We are testing the hypothesis that DNA methylation differences exist between indolent and aggressive prostate cancers and can guide functional understanding of cancer aggressiveness and enable early detection of lethal prostate cancers. Second, DNA methylation abnormalities occur in all genomic contexts throughout the cancer genome, but we know little about the functions of non-promoter DNA methylation. I envision that our findings here can have broad impact on our knowledge of how epigenetic regulation shapes the transcriptome in cancer and other biological contexts.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this profile.

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