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Charles L. Limoli

To cite this article: Charles L. Limoli (2017): Lessons learned from an unstable genomic landscape, International Journal of Radiation Biology, DOI: 10.1080/09553002.2016.1277800

To link to this article: http://dx.doi.org/10.1080/09553002.2016.1277800

Accepted author version posted online: 05 Jan 2017.
Published online: 26 Jan 2017.

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Lessons learned from an unstable genomic landscape

Charles L. Limoli

Department of Radiation Oncology, University of California, Irvine, CA, USA

ABSTRACT
Purpose: This brief historical perspective will highlight the many accomplishments of the late William ‘Bill’ Morgan, and how his laboratory during the mid-1990s shaped the field of genomic instability. Bill focused on the processes responsible for radiation-induced genomic instability, and while ionizing radiation was known to induce this phenomenon, the precise causes were poorly understood. Here we revisit Bill’s unique approach to these problems, as he advocated the use of novel mammalian cell lines to tease apart the mechanisms responsible for destabilizing an otherwise stable nuclear genome.

Conclusions: Genomic instability is a multifaceted process posited to be the driving force behind multistep carcinogenesis. Bill used a variety of innovative techniques that ultimately refined our understanding of the causes and consequences of radiation-induced chromosomal instability and the role it played in cancer predisposition. The central concepts of genomic instability fit nicely with the mutator phenotype hypothesis proposed by Lawrence Loeb, both of which represent functionally similar frameworks for describing how genomic stability can be compromised. The field of genomic instability has since advanced considerably, and much of our current knowledge is due to the efforts of Bill Morgan.

Introduction

With increasing knowledge of the intricate processes that converge to regulate the stability, replication and segregation of genomic DNA, has come an appreciation of how easily this delicate balance can be disrupted, compromising the integrity of the genome. While the myriad pathways involved provide ample opportunity for things to go awry, it is surprising that instability is not the default outcome. For that reason, it is even more important to understand how the genome can become destabilized, leading to adverse outcomes. What initiates genomic instability and then allows it to persist beyond a single cell cycle, through the life of a tissue or organism or even its transmission from generation to generation? To address these questions, we will examine the work of the late William F. Morgan, ‘Bill’ to those of us who counted him as a colleague or friend. The early definitions, observations and ideas that he put forth in the mid-1990s contributed immensely to our current understanding of the complex field of radiation-induced genomic instability.

The early years of radiation-induced genomic instability

Significant work from the laboratories of Jack Little, Tom Hei, Eric Wright and Bill Morgan shaped the field of radiation-induced genomic instability during the 1990s and subsequently. Distinct approaches tackled critical gaps in knowledge as research focused on elucidating the critical mechanisms for initiating and perpetuating the unstable phenotype. Genomic instability can be operationally defined as the increased rate of acquisition of genetic change. This all-encompassing definition includes such diverse endpoints as delayed reproductive cell death, gene amplification and mutation, and a host of karyotypic abnormalities such as the formation of micronuclei, sister chromatid exchanges and chromosomal aberrations (Morgan et al. 1996). For years, radiation exposure was known to induce these types of changes to the genome at various times soon after exposure, but evidence then started to accumulate suggesting that many of these changes persisted for many cellular generations following irradiation. Some of the first evidence for this phenomenon was generated in a series of papers from the laboratory of Jack Little, where delayed reproductive cell death was a term used to define the reduced plating efficiency exhibited in cells previously subjected to irradiation (Chang and Little 1991, 1992a). Many of these findings corroborated earlier studies from Seymour and Mothersill, documenting that clonal survivors of irradiation had high yields of lethal mutations or elevated transformation efficiencies (Seymour et al. 1986; Mothersill and Seymour 1987), indicating the propagation of radiation effects that were incompatible with more traditional views of cellular survival.

Furthermore, while evidence indicated that DNA double-strand breaks (DSB) were involved, cells that exhibited reduced plating efficiency did not contain the typical spectrum of radiation-induced deletion mutations, but rather exhibited increased levels of spontaneous mutations suggesting alternative mechanisms (Chang and Little 1992b). At about this same time, a critical paper came out from the
Wright Lab detailing the transmission of chromosomal instability after alpha-particle irradiation (Kadhim et al. 1992). The implications of this and subsequent findings from the same laboratory pointed to a mechanism(s) possibly dependent on radiation quality that was capable of propagating the memory of prior exposure to cellular progeny that had not themselves been irradiated (Kadhim et al. 1994, 1995).

The search for and characterization of radiation-induced genomic instability

Many of these early observations prompted a number of labs to investigate the various mechanisms that might contribute to the propagation of radiomimetic signatures across multiple generations. At this time, significant work focused on the mechanisms by which cells correctly and incorrectly rejoined broken DNA was being conducted by Bill Morgan then at the former Laboratory of Radiobiology and Environmental Health (LREH) at the University of California, San Francisco.

Bill was uniquely poised to address a number of critical questions in the field of genomic instability. This in large part was due to his implementation of a hamster-human hybrid line modified to contain a single copy of human chromosome 4 and for developing the methodology of electroporating restriction endonucleases into mammalian cell lines in order to study DNA repair mechanisms (Winegar et al. 1989; Morgan and Winegar 1990). These enabling technologies allowed one to follow multiple rearrangements of human chromosome 4 in a background of hamster chromosomes by means of fluorescence in situ hybridization, and to follow the fate of specific DSBs produced by restriction cleavage. With these tools at his disposal, Bill and his co-workers were able to demonstrate that exposure to ionizing radiation resulted in significant yields of surviving subclones exhibiting delayed chromosomal instability (Marder and Morgan 1993) and that the production of relatively simple DNA DSBs resulted in illegitimate recombination events leading to a large spectrum of deletion mutants (Phillips and Morgan 1994).

Since DNA DSBs were then widely accepted as the critical radiolytic lesions responsible for radiation-induced cell kill, Bill passed on a project to the author of this paper that involved generating subclones expanded from single cells surviving restriction endonuclease treatment, to determine the frequency of resultant chromosomal instability. An eager postdoc at the time, I proceeded to analyze over 60,000 metaphases generated from over 300 clones subjected to four different restriction endonucleases to discover that none of these treatments had produced a single clone showing evidence of a destabilized genome. As an afterthought, we surmised that restriction enzyme-induced DSBs were poorly radiomimetic, and that agents producing DNA end groups more similar to those produced by ionizing radiation might lead to detectable genomic instability. In the end, radiomimetic agents such as bleomycin and neocarzinostatin were found to be relatively efficient inducers of chromosomal instability, while agents producing readily ligatable end groups (restriction endonucleases) or singly damaged sites (hydrogen peroxide) were far less efficient at or completely unable to eliciting an unstable phenotype (Limoli et al. 1997b).

We quickly recognized that a major limitation to moving this line of investigation forward was the lack of readily available genomically unstable cell lines. This prompted extensive work aimed to generate an arsenal of chromosomally unstable and stable clones. The approach taken in Bill’s lab was to expose the hamster-human hybrid cell line to a range of radiation doses (2–10 Gy), isolate survivors and proceed to characterize expanded subclones for the presence or absence of chromosomal instability. The analysis of nearly 700 subclones and more than 140,000 metaphases yielded countless chromosomally stable subclones and a dozen or so ‘holy grails’, or subclones that exhibited both marked and dramatic chromosomal instability (i.e. those clones possessing at least three or more distinct metaphase subpopulations of chromosomal aberrations in at least 5% of the total metaphases scored). From these extensive studies we were able to demonstrate a bona fide dose response, where the incidence of chromosomal instability was 3% per Gray (Limoli et al. 1999), an incidence that increased to 4% per Gray following heavy ion irradiation (Limoli et al. 2000). Interestingly, these studies also included the irradiation of GM10115 cells substituted with bromodeoxyuridine (BrdU), known to be one of the only true chemical radiosensitizers. Subclones substituted with BrdU showed a dramatic increase in the frequency of chromosomal instability that reached a plateau in the low dose region (2 Gy), providing further evidence for a nuclear target for the induction of genomic instability.

The undeniable legacy of the foregoing studies from the Morgan laboratory was the stockpile of chromosomally stable and unstable subclones that were generated and that provided the foundations for additional investigations. Over the ensuing years many of these subclones were distributed to a number of labs that have since corroborated their utility as useful reagents for studies focused on deciphering the mechanisms underlying radiation-induced genomic instability.

The critical radiolytic target for genomic instability

While certain data sets implicated DNA as the critical target for radiation-induced genomic instability, others did not. Some of the more convincing evidence suggesting non-nuclear targets came from studies implementing alpha-particle irradiation, where the number of particle traversals could be correlated to the number of ‘hits’ per cell. These data revealed much higher proportions of cells surviving and exhibiting chromosomal instability than predicted based on Poisson statistics, and argued that nuclear traversals were not necessary to transmit the unstable phenotype to the surviving unirradiated descendants of irradiated cells (Lorimore et al. 1998). Since that time, additional elegant work using microbeams at the Radiological Research Accelerator (RARAF) facility at Columbia has shown that cytoplasmic traversals suffice to elicit various indications of genomic instability (Hu et al. 2012). The mechanisms proposed involve mitochondrial dysfunction and elevated reactive oxygen and nitrogen
species that could initiate a lipid peroxidation signaling pathway involving 4-hydroxynonenal and cyclooxygenase-2 (Hong et al. 2010; Zhang et al. 2013). Many of these mechanisms lay at the heart of ‘bystander’ or ‘non-targeted effects’, which became a primary interest of Bill’s once he left LREH at UCSF and became the Director of Radiation Biology at the University of Maryland. While an extensive review of the bystander literature is beyond the scope of this article, Bill was instrumental in promoting the importance of this field to low dose radiobiology and its relevance to occupational exposure risk. Bill co-authored a number of excellent primary research papers relevant to this topic (Morgan et al. 2002; Huang et al. 2004) as well as several focused and extensive reviews (Morgan 2003a, 2003b, 2011; Morgan and Sowa 2007, 2009, 2015).

Oxidative stress and mitochondrial dysfunction

With significant data implicating a number of potential critical intra- and extranuclear targets for the propagation of genomic instability to the progeny of surviving cells, the field during the late 1990s was still searching for a more unifying explanation that could account for the seemingly disparate observations measured in a wide range of in vitro model systems. One often overlooked paper provided several initial clues about arguably the most fundamental and persistent biochemical perturbations brought on by ionizing radiation exposure – oxidative stress (Clutton et al. 1996). The idea that oxidative stress and resultant injury might provide a potential biochemical mechanism for both initiating and perpetuating the various endpoints of genomic instability was compelling and immediately appealing, prompting a series of studies from Bill’s lab and later from my own, aimed at substantiating this hypothesis.

With the power of having our previously generated ‘arsenal’ of unstable and stable hamster-human hybrid subclones, work started to directly compare these phenotypically distinct subsets of clones for other indications of genomic instability and oxidative stress. Side-by-side comparison between these chromosomally stable and unstable subclones revealed that unstable clones had increased levels of delayed reproductive cell death, gene amplification, apoptosis, increased levels of intracellular ROS and RNS, and increased levels of lipid peroxidation end products (Limoli et al. 1997a, 1998). Further support for the idea that oxidative stress was involved in the initiation and perpetuation of genomic instability came from a series of follow up studies demonstrating that free radical scavengers present during irradiation could attenuate the subsequent onset of radiation-induced genomic instability (Limoli et al. 2001), and that chromosomally unstable clones exhibited a marked and persistent oxidative stress linked to mitochondrial dysfunction (Limoli et al. 2003). The importance of chronic as opposed to acute oxidative stress was also found to be critical, as the induction of chromosomal instability in cells subjected to steady state levels of hydrogen peroxide was quite high in the progeny of surviving cells (~20%) compared to those treated with an acute bolus of this agent (Limoli and Giedzinski 2003). Other labs also demonstrated a link between radiation exposure and perturbed metabolism, finding evidence for persistently increased generation of reactive species linked to mitochondrial damage (Leach et al. 2001). Related work with the Bill’s stable and unstable subclones showed a causal relationship between the mutator phenotype in chromosomally unstable clones and elevated hydrogen peroxide (Dayal et al. 2008). Data indicated that this was likely due to defects in the function and/or assembly of mitochondrial electron transport chain complex II, ultimately elevating hydrogen peroxide levels capable of contributing to genomic instability through the production of hydroxyl radical-induced DNA DSBs generated through Fenton chemistry (Dayal et al. 2009; Owens et al. 2012). Interestingly, proteomic analysis of one of the more unstable hamster-human subclones corroborated the foregoing data, finding that proteins involved in the tricarboxylic acid cycle are upregulated to compensate for various defects in mitochondrial electron transport (Thomas et al. 2012).

The Morgan legacy: laying the foundation for future research endeavors

Work from Bill’s laboratory has paved the way for many to pursue the radiation sciences. His findings, teachings, and enthusiasm have inspired his colleagues, postdoctoral scholars and students to continue his legacy and develop new areas dedicated to a deeper understanding of radiation effects using a variety of biological systems. Genomic instability is now widely accepted as contributory if not causal to carcinogenesis, and from its early inception, has grown to encompass a wide range of scientific disciplines dedicated to an understanding of the complexities of what goes wrong during the transformation of a normal cell to a cancerous cell. The essence of many of these ideas are captured in Figure 1, depicting Bill amongst the clonal heterogeneity arising from a single irradiated progenitor cell that ultimately gives rise to the many phenotypic endpoints associated with genomic instability.

We now know that coordinating the activation of the DNA damage response to the inhibition of cell cycle progression and DNA repair are critical in preventing the propagation of cells harboring dangerous mutational loads. Replication stress likely provided some of the driving force behind the evolution of DSB repair, as well as checkpoint control during S-phase. Genomic instability is routinely associated with an array of biochemical perturbations that influence metabolic transitions and disrupt electron transport, invariably elevating the levels of reactive species in cells. The resultant oxidative stress drives additional mutations and plays an important role in mitochondrial function that ultimately provides the substrates required for nearly every epigenetic modification. The importance of epigenetic modifications to DNA and the chromatin have been appreciated for decades, and may soon be recognized as the principal mechanism for maintaining the integrity of cellular genomes and to those passed on to cellular progeny. Heritable effects of radiation are most certainly linked to...
epigenetic states, and unraveling the epigenetic code may provide new insights into the regulatory controls that prevent genomic instability from becoming a dominant trait. Many of the foregoing issues were heated points of discussion in the Morgan lab, fueled by select antioxidants, lively discourse and an atmosphere that promoted the essence of the scientific process. Bill’s impact on the radiation science community is indisputable and his impact on friends and family even more certain. He will be missed.

Take care my friend.

Disclosure statement
The author reports no conflict of interest. The author alone is responsible for the content and writing of the paper.

Funding
This work was supported by HDTRA 1-13-1-0022 (CLL).

References

Figure 1. The Morgan Legacy. Professor Morgan shown within a backdrop of the concepts he helped discover and develop regarding radiation-induced genomic instability.