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Editorial

Cancer research in an era when epigenetics is no longer "epi" —challenges and opportunities

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When I was learning epigenetics 17 years ago at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, this field was like a small spring bud growing in the windy storm of genetics/genomics. Now, epigenetics is booming like gold mining in the American West, and every cancer scientist intends to make a fortune from it. When epigenetics eventually becomes a mainstream, or even leading, research field in biomedicine[1] and is no longer "epi", what else should we say or what precautions should we take?

Several years ago, a cancer professor told me that he would not trust the RASSF1A methylation reports because the published data ranged from 30% to 80% methylation in tumor samples of the same tumor type. What should we do when the specific and sensitive methylation-specific polymerase chain reaction (MSP) detection, which is now used everywhere, can also yield significant non-specific detection rate if the primers are not properly designed (e.g., for ATM)[2]? I have read and reviewed manuscripts, even from leading mainstream journals, detailing experiments in which the MSP primers used for the detection of methylation of tumor suppressor gene (TSG) promoters contained only one CpG or even none at all, thus most likely making the primers non-specific and resulting in false-positives. Some studies have used 10–20 times more bisulfited DNA template for TSG methylation analysis than is used in our lab's system (Table 1)[3], which is more or less the same as that used by most methylation labs at Johns Hopkins [4]. I have also seen reports of studies using nested MSP to detect promoter CpG methylation in primary tumors in which the researchers obtained 100% positivity for TSG methylation. Some studies have focused on the wrong genomic region, which has no relevance to the regulation of the studied gene [5], although we know that the critical regulatory region (which may contain exon 1) of a gene and a gene's body (introns and other exons) have completely different methylation profiles and most likely different functional roles. Is it sufficient to detect the methylation status of just 1 or 2 CpG sites of a given gene, even when high-throughput screening approaches can simultaneously examine thousands of genes in a sample? There still seems to be some way to go before "standard methylation analysis" can be formalized and performed properly and efficiently. Nevertheless, epigenetic study has been an effective and specific way to discover critical cancer genes, especially novel TSGs[6], a process that used to consume decades of work for classic geneticists. For example, using epigenetic approaches, we have discovered a series of novel TSGs, including the long-sought 1p36 tumor suppressor gene CHD5 which codes for an important epigenetic modifier.

Meanwhile, the translation of "epigenetics" into Chinese is also peculiar and used to be quite confusing (with ~10 different translations in use). Although now the common term is "表观 (biaoguan) genetics", many scientists do not like this translation because the Chinese characters "表观" mean "superficial" or "on the surface" (it was previously felt that CpG methylation was on the surface of DNA molecules, and thus "epigenetics" was translated as "表观"). How does this translation reflect the numerous different types of histone modifications, as well as RNA inference, which are mostly "deep inside" epigenetic features? When the National Natural Science Foundation of China (NSFC) Agency set up a specific "epigenetics task" fund of ~¥400 million in 2006, it was said that all of a sudden hundreds of new "epigenetic" research groups in China came up to apply for this "epi-" fund. Is it really an "epi" phenomenon in biomedicine research?

When whole-exome sequencing (WES) or even whole-genome sequencing (WGS) becomes affordable for most key labs and when more and more WES/WGS papers are published, we should still bear in mind that many critical TSGs have only infrequent genetic mutations and are disrupted predominantly by epigenetic mechanisms (epi-mutations) in human cancers. These epi-mutations will still be missed even when the whole cancer genome is sequenced base by base. We believe that genomics and epigenomics should be beautifully integrated to obtain a complete picture of the human

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Table 1. The methylation-specific polymerase chain reaction (MSP) system with Taq-Gold enzyme used in our lab

MSP system component	For a 25 μL reaction	Final Concentration
10× PCR buffer II (PE)	2.5 μL	1×
25 mmol/L $MgCl_2$ (PE)	2 μL	2 mmol/L
dNTPs (2.5 mmol/L each)	2 μL	0.2 mmol/L
5′ primer (10 μmol/L)	1.5 μL	0.6 μmol/L
3′ primer (10 μmol/L)	1.5 μL	0.6 μmol/L
Taq-Gold (5 U/μL) (PE)	0.125 μL	1.25 U / 50 μL
dd H_2O	14.375 μL	
Bisulfited DNA*	1 μL (50 ng)	0.1 μg /50 μL

*1 μg of DNA is bisulfite treated and dissolved in 20 μL of TE buffer and then stored at –20℃. MSP was performed at 95℃ for 10 min, followed by 40 cycles of 94℃ for 30 s, annealing at ~58–60℃ for 30 s, 72℃ for 30 s, and then ended at 72℃ for 5 min.

cancer gene profile, a process that is being carried out currently for most common cancers[7].

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