Nuclear Factor One transcription factors as epigenetic regulators in cancer

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Abstract

Tumour heterogeneity poses a distinct obstacle to therapeutic intervention. While the initiation of tumours across various physiological systems is frequently associated with signature mutations in genes that drive proliferation and bypass senescence, increasing evidence suggests that tumour progression and clonal diversity is driven at an epigenetic level. The tumour microenvironment plays a key role in driving diversity as cells adapt to demands during tumour growth, and is thought to drive certain subpopulations back to a stem cell-like state. This stem cell-like phenotype primes tumour cells to react to external cues via the use of developmental pathways that facilitate changes in proliferation, migration, and invasion. Because the dynamism of this stem cell-like state requires constant chromatin remodelling and rapid alterations at regulatory elements, it is therefore of great therapeutic interest to identify the cell-intrinsic factors that confer these epigenetic changes that drive tumour progression. The Nuclear factor one (NFI) family are transcription factors that play an important role in the development of many mammalian organ systems. While all four family members have been shown to act as both oncogenes and tumour suppressors across various cancer models, evidence has emerged associating them as key epigenetic regulators during development and within tumours. Notably, NFIs have also been shown to regulate chromatin accessibility at distal regulatory elements that drive tumour cell dissemination and metastasis. Here we summarise the role of the NFIs in cancer, focusing largely on the potential mechanisms associated with chromatin remodelling and epigenetic modulation of gene expression.

Introduction

Epigenetics has historically been defined as heritable cellular phenotypes of organisms that are independent of alterations in DNA sequence, however as the term has continued to evolve, it is now used more to describe changes in cellular and molecular phenotype that are associated with alterations to chromatin structure and accessibility[1, 2]. A major substrate of epigenetic change is chromatin, the macromolecular complex made up of DNA and histone proteins. Chromatin can be modified by four distinct mechanisms; these are DNA methylation, histone modification, nucleosome remodelling and RNA-mediated targeting. While epigenetic processing was first described as an important process in development[3], its misregulation is now thought to also be a key component in driving cancer formation and tumour progression[4, 5]. For example, factors associated with tumour progression such as aging[6], chronic inflammation[7] and environmental exposure such as to cigarette smoke[8] alter the epigenome of cells.

One key aspect associated with cancer progression is tumour heterogeneity[9]. This heterogeneity is not only defined by the various cell types often found within tumours (fibroblasts, endothelial cells, pericytes, and immune cells), but also at the genetic and phenotypic level within individual cancer cells[10]. Despite many tumours originating via irreversible mutations in oncogenes and tumour suppressors derived from a single cell, many cancers display large levels of clonal diversity[11]. While the mechanisms behind tumour heterogeneity at the genetic and phenotypic level are continually being defined, there is a clear epigenetic component that drives this process[12]. The highly proliferative nature of many malignancies makes the tumour microenvironment very dynamic during growth and as such, cells must continuously adapt to environmental stressors. Conditions such as nutrient deprivation, hypoxia, immune responses, and even treatments aimed at destroying tumours have been shown to induce considerable changes in cancer cell DNA methylation status and chromatin remodelling[13, 14]. Cross talk between cellular populations (both cancer and other stromal populations), either through direct interaction, or via secretory factors, can also induce changes in cell motility and proliferation at an epigenetic level. An interesting observation commonly associated with cellular invasion is the idea that many cancer cells undergo a process of de-differentiation to attain a stem cell like phenotype[15, 16]. Such a cell state is extremely dynamic and primed to respond to external cues, allowing it to

quickly alter its proliferative and migratory characteristics throughout various developmental stages[12, 17]. This dynamism relies heavily on chromatin remodelling and changes to histone structure and function, through the combinatorial effect of a myriad of histone modifications that can be rapidly altered to facilitate immediate and reversible changes to gene expression[18].

Micro RNAs (miRNA) are another key epigenetic regulator that potentiates tumour progression. They can act as either oncogenes or tumour suppressors and as such, can be regulated in a manner that their expression is increased or decreased via transcriptional regulation. There are various mechanisms contributing to miRNA deregulation in cancer cells[19]. Interestingly, while the regulation of cancer cell phenotypes via miRNAs is often an intrinsic cellular process, there are various mechanisms in which miRNAs can be secreted in the microenvironment to induce autocrine and paracrine effects on other tumour cell populations[19]. They can also play a distal role in regulating tumour angiogenesis, immunophenotype, and extracellular matrix (ECM) remodelling[19, 20]. Deregulation of miRNAs within stromal cell populations has also been shown to play a large role in promoting initial tumour formation and progression[20], while other microenvironmental factors such as hypoxia, nutrient deprivation, and increased acidity also drive aberrant miRNA expression in cancer cells [21, 22].

Tumour heterogeneity has a clear therapeutic impact[23-25]. The clonal diversity observed in many developed tumours is vast and frequently small clonal subsets will confer selective advantages that drive tumour progression or remain refractory to therapies that are effective against the majority of cells in the tumour. It is clear that modulation of the genome at the epigenetic level plays a major role in heterogeneity and is therefore of great therapeutic interest to identify key regulators that confer these epigenetic changes throughout tumour progression.

This review will focus one such family of regulators, the NFIs, and their role in tumour progression. First described as a host protein required for adenovirus replication (Nagata et al., 1982), there have been four NFI transcription factors identified in vertebrates, NFIA, NFIB, NFIC and NFIX[26, 27]. NFIs are developmentally important proteins that in broad terms drive progenitor cell

differentiation within the central nervous system[28-30], lung[31, 32] and muscle[33, 34]. After early embryonic and postnatal development, NFIs continue to have important roles in regulating progenitor cell biology. In adult progenitors cells, within neural tissue, the skin, and skeletal muscle, NFIs promote differentiation[35, 36] but also regulate the balance between cell-cycle entry and exit[36-38], and thereby regulate the homeostasis that exists between progenitor pool expansion and tissue regeneration. NFIs have also been established as key tumour suppressors and oncogenes across a range of cancers, with recent evidence suggesting that they may function as key drivers of tumour progression by coordinating changes in the epigenome[39].

NFIs as epigenetic regulators

Transcription factors are traditionally defined by their ability to coordinate gene expression by binding directly to regulatory regions within DNA alone, or via protein interactions with transcription co-factors that bind DNA indirectly through interaction with transcription factors at cis-regulatory sites. NFIs for example, can both activate or repress gene expression by direct binding to the DNA dyad symmetric consensus site or half-site sequence TTGGC(N5)GCCAA on double stranded DNA[40-42], forming homodimers or heterodimers with other proteins. While this is a key feature of transcription factors, evidence reveals that many families are able to alter gene expression by modulating chromatin structure directly through nucleosome remodelling, or indirectly via interactions with, or the regulation of, epigenetic modifiers. We argue that this may also be true of NFI family members, consistent with a number of studies demonstrating that NFIs interact with chromatin and chromatin regulators in a variety of ways. Moreover, recent chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq) experiments suggest that the net effect of these NFI-chromatin interactions may be to increase chromatin accessibility and gene expression [39, 43].

NFI activity generally correlates with increased expression of their target genes and are associated with higher levels of active promoter methylation marks such as H3K4me3 and H3K36me3, implicating them predominantly as activators of transcription[44]. The first evidence implicating the NFI family acting in a transcriptionally independent manner found that they were able to bind to GCCAAT recognition sites and serve as initiation factors in DNA replication[45]. Moreover, early structural analysis of NFIs identified their potential for chromatin regulation as they contained a trans-activation domain that interacts with histone H1 and H3[46, 47]. Further functional analysis demonstrated that NFIs were able to alter the interaction of reconstituted nucleosomal cores with DNA *in vitro* in a growth factor-dependent manner[47]. *In vivo* evidence also revealed that NFIs are able to alter native chromatin structure at yeast origins of replication[48] and other promoter regions through direct interaction with histone proteins[49]. NFIs were also shown to activate simian virus 40 (SV40) DNA replication *in vivo* by interacting directly with histone H3 and relieving nucleosomal repression at the SV40 origin[50]. Indeed, there are many other examples of NFI directly interacting with the nucleosomal architecture. Studies in yeast cells

for example, show that NFI was able to delimit chromatin domain boundaries by binding histone H3, thereby preventing epigenetic teleomeric silencing by forming a partition between the gene and telomere and blocking silent information regulator (SIR) proteins involved in the de-acetylation of histone tails[51]. More recently, genome wide mapping analysis of NFI DNA binding sites within mouse embryonic fibroblast confirms that there is a clear association between NFI and histone H3[44]. Consistent with this, NFI globally associates with chromatin domain boundaries, separating permissive and silent chromatin markers as defined by localisation with H3K27me3 and H3K36me3 boundaries of opposite polarities, and they were further found to directly interact with positioned nucleosomes[43].

Crucially, NFIs have also been shown to interact with chromatin in human cells. NFIs are able to prevent the silencing of transgenes upon chromosomal integration in a histone dependant manner, however they were unable to activate transcription alone in HEK293 cells[52]. Studies performed in HeLa cells investigating mechanisms underlying the boundaries between euchromatic (active, permissive chromatin) and heterochromatic (silent, condensed chromatin) domains confirmed that NFI proteins, or fusions containing the histone binding domain of NFIs, can partition two genes colocalized at a telomeric locus into active and inactive chromatin structures[53]. These findings suggest a mechanism whereby NFI interacts directly with nucleosomes in a transcriptional independent manner to establish a chromosomal structure that blocks silencing signals emanating from the telomere, while maintaining a permissive chromatin state to allow for increased gene expression within these regions. It also provides further mechanistic evidence to explain the previous findings that NFIs can induce a permissive chromatin state to reverse chromatin-mediated gene silencing without being able to activate transcription of these genes alone[52].

In depth analysis of NFI interactions with chromatin and their role in remodelling have also been performed using long terminal repeat (LTR) regulatory sequences of the mouse mammary tumour virus (MMTV) (for an in depth review on this, see[54]). NFI-binding sites were found on B nucleosomes in the MMTV promoter in the 1980s, with research showing that there is a functional synergism between NFI and the glucocorticoid receptor (GR) in binding to a chromatin template and activating the promoter, which was abrogated in a nucleosome depleted environment[55]. Follow up

studies in this model system using linker-scanning mutants of transcription factor binding sites found that NFIs were required for hormone-dependent chromatin remodelling, as they found that binding site mutations substantially decreased hormone-mediated remodelling of nucleosome B and that NFI was also necessary for the association between the BRG1 chromatin remodelling complex and GR on the promoter *in vivo*[56]. Studies into *S. cerevisiae* looking at minichromosomes assembled on MMTV LTR are consistent with a role for NFIs in chromatin remodelling, as they act as classical transcription factors in a relaxed chromatin context, whereas in a wild type chromatin confirmation, NFI cooperates with steroid hormone receptors to stabilize an open chromatin confirmation by interacting with the nucleosome[56]. Moreover, *in vivo* studies using *Xenopus* oocytes uncovered a role of NFIs in initiating nucleosome remodelling independent of these chromatin-remodelling complexes[57]. NFIs have also been shown to interact with Octamer Factor 1 (OCT1) and form a present chromatin structure through remodelling[58]. This study found that both transcription factors were able to bind to their cognate sites within the MMTV promoter to alter chromatin structure from randomly positioned nucleosomes to induce a partial translational positioning that enhances the hormone-induced response of the receptor and correlates with increased basal transcription[58]. Overall these model systems have defined a clear role where NFIs can act in a transcriptionally independent manner to regulate gene expression by altering chromatin structure through direct remodelling and induce a permissive chromatin state via nucleosomal interaction.

NFIs have also been shown to affect chromatin remodelling in a more indirect manner by regulating the expression of numerous chromatin-modifying genes and by interacting with chromatin modifiers. The Enhancer of zeste homolog 2 (EZH2) is a histone-lysine N-methyltransferase enzyme that represses transcription by catalysing the addition of methyl groups to histone H3 at lysine 27. NFIB has been shown to directly repress EZH2 during development of the cerebral cortex to promote neurone differentiation[59]. The genome-wide mapping study previously discussed by Pjanic and colleagues[43] correlated NFI with histone methylation modifications H3K4me3 and H3K36me3 at markers of transcribed genes and even outside annotated transcribed loci, suggesting NFIs may facilitate these modifications. NFIs have also

been show to interact with histone deactylases such as BAF at various promoter regions[60, 61] and other transcriptional activators such as BRG1[56].

Epigenetic mechanisms of NFIs in cancer

In light of these findings, it is clear that the NFI family have dual functionality in acting as classical transcription factors, but also as epigenetic modifiers. In addition to development and maintenance of normal cellular physiology, NFIs have been shown to act as tumour suppressors and oncogenes across a wide range of cancer types (see Table 1 for a brief overview). Within the context of cancer, chromatin accessibility studies are regarded as one of the most relevant genomic characteristics that correlate with activity at specific loci, and efforts to characterize changes in global chromatin structure between normal cells, cancer cells and lines differing in metastatic potential have become a considerable focus of cancer research [62-64]. Recent high-impact studies examining NFIB function in small cell lung cancer (SCLC) suggest that in this context, NFIs might modify chromatin architecture in a manner that results in increased tumour heterogeneity[39, 65]. The most prominent study implicating NFI as an epigenetic driver of cancer progression found that NFIB promotes metastasis through a wide spread increase in chromatin accessibility[39]. In this study, primary and metastatic tumours were isolated from genetically engineered mice, and genome-wide characterization of chromatin accessibility (ATAC-seq) was used to identify global changes throughout the different tumours. These studies demonstrate that there was a dramatic increase in chromatin accessibility that occurs during malignant progression. The most highly enriched motif identified between the differentially accessible regions was the binding site for the NFI family, with the hyper-accessible samples also showing genomic amplification of the NFIB locus. Crucially NFI motifs in hyper-accessible samples had increased promoter occupancy, depleted nucleosomes, and exhibited a significant change in local chromatin architecture, the maintenance of which was dependent on NFIB expression. While the mechanistic basis of the NFIB driven hyperaccessibile chromatin state was not reported in this study, the data was suggestive of SCLCs containing a "preset" configuration during early malignant transformation that permits initial binding of these motifs following NFIB up regulation. This proposed mechanism suggests an intrinsic NFI driven signal can promote metastasis irrespective of genetic mutation

and warrants further investigation into other cancer subtypes. Analysis of DNA footprinting showed NFIB had a long half-life upon binding to consensus sites and it was suggested that this long residency allows adaptation of nearby chromatin to enforce an opened architecture, which is further stabilized by binding of other motifs to the enriched regions in the newly open sites. Analysis of NFIB interacting directly with histones H1 and H3 or recruiting chromatin-modifying complexes was not investigated but was suggested to potentially complement the study's proposed mechanism. Further research investigating these potential interactions would provide a greater mechanistic insight of NFIs in remodelling chromatin within a cancer context, and may provide scope for therapeutic targets against chromatin modifiers.

A potential tumour suppressor role has also been identified for NFIA and NFIC in breast cancer through the epigenetic repression of a disintegrin and metalloprotease domain-containing protein 12 (ADAM-12)[66]. ADAM-12 levels in urine have been correlated with disease progression in breast and bladder cancer, have been shown to be up regulated in many cancers, and also promote cancer metastasis. The chromatin modifier methyl CpG binding protein 2 (MeCP2) is a transcriptional repressor that is associated with DNA methylation, histone deacetylation, and with the recruitment of chromatin remodelling complexes[67-69]. Ray and colleagues[66] identified a novel interaction between NFIs with MeCP2 at Z-DNA elements of ADAM-12 to induce its repression. Z-DNA forming regions and promoter sites for NFI have previously been highly correlated with the transcriptional start site of many genes[70], and this study demonstrated that the MeCP2-NFI interaction at Z-DNA elements is necessary for repression, and that MeCP2 deficiency in breast cancer cells results in ADAM-12 overexpression and tumour progression[66]. Another study focusing on the epigenetic regulation of the tumour suppressor gene hDAB2IP in prostate cancer lines found that there was an increase in acetylhistone H3 levels associated with the promoter in PCa prostate cancer lines[71]. ChIP assays identified several NFI binding motifs associated with this region, potentially implicating a functional role in nucleosomal binding of NFIs in this cancer context.

In addition to altered expression, numerous examples of NFI fusion proteins have been reported in a number of cancer subtypes. Cases have been documented across

acute erythroid leukaemia, adenoid cystic carcinomas, breast cancer, and pilocytic astrocytoma, all with different protein fusion targets[72-76]. While the prevalence of such changes is well documented, the functional nature of these lesions has yet to be explored. Consistently, NFIs as fusion proteins appear to increase the activity of their respective fusion partners' downstream signalling pathways, but do not appear to effect NFI regulatory target gene expression[72, 73]. Fusion proteins have been reported with transcription factors (NFIB-MYB), chromatin modifying complexes (NFIA-CBFA2T3), and kinases (NFIA-RAF1) and usually contain only a partial form of NFI[72, 74, 75]. Studies discussed previously in this paper highlight NFIs role in forming a chromatin domain barrier/boundary that blocks propagation of silencing signals emanating from heterochromatic silent regions within the telomere[53]. The formation of these domains allowed NFI to maintain a permissive open chromatin state upon binding to histone regions, which allowed for active gene expression within that area. Esnault and colleagues (2009) found that only the histone-binding domain of NFI was necessary to perform this function. They employed the use of NFI fusion proteins with GAL4 and found that they performed this barrier function irrespective of the reporter gene identity or its transcriptional orientation and distance from the promoter, and also did not affect genes adjacent to the telomere. These findings highlight the potential of NFI genes implicated as fusion proteins in cancer to remodel chromatin areas in a transcriptionally independent manner and to allow a permissive open chromatin state for increased binding of their fusion counterpart to regulatory genomic regions.

Conclusions

Genome wide analysis of global changes in chromatin landscape and architecture within cancer is a relatively new field. There is great scope for studies investigating NFIs as potential chromatin regulators at both a global and local level across various cancers, as they have clearly been implicated as epigenetic modifiers during development and homeostasis. The study conducted by Denny and colleagues[39], is one of the first showing that a single transcription factor is capable of inducing chromatin state changes globally upon high expression, and also highlights the capability of hyperaccessible genomic regions in driving a metastatic phenotype[39].

The finding that there appears to be a 'preset' configuration in malignant transformed cells for the binding of NFI motifs to induce chromatin hyperaccessibility shows that NFIs are key in inducing the switch necessary for metastatic progression in SCLC. It is highly suggestive of a novel, pre-defined intrinsic mechanism in driving metastasis in cancer cells that occurs at an epigenetic level, as opposed to tumour cells progressing via the accumulation of genetic mutations from genomic instability. Given that this work only focused on SCLC, it remains to be determined if similar changes in chromatin accessibility driven by NFIs is relevant in other cancer subtypes, or whether other transcription factors are capable of a similar function. Certain events imposed on cells by the tumour microenvironment (hypoxia, nutrient deprivation, increased acidity etc.) are key drivers of tumour heterogeneity[12] and may be capable of driving changes in NFI expression and as such, may have the potential of inducing this hyperaccessible state following increased NFI expression. This warrants further investigation into the potential heterogeneity of NFI transcription factor expression and cellular function across various tumours, and whether the microenvironment is capable of altering NFI expression.

Future studies using single cell sequencing approaches will provide improved resolution as to whether NFIs drive epigenetic changes uniformly across cellular populations, or if this differs vastly on a per cell basis. For example, there exists the potential for single cell ATAC-Seq technology as an approach for profiling single cells at an epigenetic level[77]. Given the cell-cell variability within tumours and the potential for a pre-set epigenetic state to drive metastasis, this methodology may be useful in further uncovering epigenetic changes associated with heterogeneity and metastatic progression. These data can be enhanced further by the addition of expression profiling of individual cells by Drop-Seq analysis, where thousands of single cells, originating from dissolved *in vivo* tissues are forced into miniscule droplets before RNA-Seq analysis[78]. If this technology can further extend to ATAC-Seq or histone modifications, it would become a useful tool in further defining epigenetic differences in heterogenous tumour populations.

The mechanism through which NFIs promote chromatin accessibility in SCLC and potentially, other cancers, remains to be defined. A fascinating proposition could be that like in normal cellular physiology, NFIs function to inhibit silencing signals

emanating from telomeric ends[51-53]. Telomeres and associated proteins form a unique DNA-protein structure that protects chromosome ends from being recognised as DNA double stranded breaks, and are well documented tumour suppressors[79]. As mentioned, telomeres have previously been thought to regulate proximal gene expression, as the heterochromatic nature of their structure coupled with the recruitment of SIR and other histone deacetylase (HDAC) enzymes results in the short range spreading of a hypoacetylation signal[53]. A recent study however, has demonstrated that chromosome looping can induce telomeric silencing up to 10 Mbs away from the telomere, implicating their role in the regulation of a large number of genes at each chromosomal end[80]. The potential genes and the significance behind their regulation during homeostasis and within cancer has yet to be defined, however given NFIs previously defined mechanism, one would predict to see an increased accessibility of chromatin and expression of genes close to telomeric regions in SCLC if they displayed this functionality. Evaluation of existing ATAC-seq and RNA-seq datasets in SCLC[39] could provide this answer. Given NFIs ability to affect chromatin structure in a multitude of ways however, it is important to consider that other mechanisms (both direct and indirect) may be key in driving this hyperaccessible phenotype.

Research implicating the importance of epigenetics in cancer has made chromatin-modifying enzymes an attractive therapeutic target. Several HDAC and DNA methyltransferase (DNMT) inhibitors are undergoing clinical trial and are now recognised as effective and well-tolerated treatments both alone and in combination with other therapies[81-83]. Transcription factors are notoriously difficult to target therapeutically, and despite recent advances[84], targeting of NFI proteins may be challenging in certain tumour contexts. NFI members are very similar in terms of sequence homology and DNA binding and there are several documented cases where certain members act in opposite tumourigenic roles [66, 85, 86]. Given the extensive history of NFIs acting as chromatin regulators during development and homeostasis, studies investigating a similar role for NFIs within cancer may highlight potential therapeutic candidates downstream that may be targeted more effectively. The potential for NFI to alter global chromatin architecture within a subset of cancers to drive metastasis may also a make it a great prognostic marker for tumour progression.

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Table.1 NFI family in cancer

Cancer type	Gene	Oncogene/Tumour suppressor	Reference
Acute erythroid leukaemia	NFIA	Oncogene, forms NFIA/CBFA2T3 fusion protein	75
Adenoid cystic carcinomas	NFIB	Oncogene, forms a fusion protein with MYB oncogene	72, 73
Breast cancer	NFIA	Oncogene and tumour suppressor, regulates SULT1A1 levels, increases cancer risk, drug resistance. Inhibits ADAM12 expression	66, 72, 76, 85-88
	NFIB	Oncogene, associated with HER2 overexpression and survival.	
	NFIC	Tumour suppressor and oncogene, suppresses EMT, migration, and invasion through KLF4-E-cad axis. Also regulates SULT1A1 levels. Inhibits ADAM12 expression	
	NFIX	Oncogene, forms fusion protein with Mast kinase, promotes proliferation.	
Cutaneous squamous cell carcinoma	NFIB	Tumour suppressor, targeted by onco-miR-365	89
Cervical carcinoma	NFIA NFIB NFIC NFIX	Oncogene, NFI-SKI interaction drives TGF-β inhibition in HPV16, drives tumour progression.	90

Diffuse gastric cancer	NFIX	Tumour suppressor, NFIX potentially binds to CDH1 risk polymorphism.	[91]
Oesophageal squamous cell carcinoma	NFIA	Oncogene, drives proliferation and migration	[92, 93]
	NFIX	Tumour suppressor, MIR-1290 targets NFIX, promotes proliferation, invasion.	
Glioblastoma	NFIA	Oncogene, down regulates P53, P21, and PAI1 to drive growth and migration.	[94, 95]
	NFIB	Tumour suppressor, associated with improved survival, ectopic expression inhibits tumourigenesis	
Glioma	NFIA	Oncogene, Inhibits p21 required for tumourigenisis	[96]
Hepatocellular carcinoma	NFIB	Oncogene, up regulated in HCC and drives survival	[97]
Lung adenocarcinoma	NFIB	Tumour suppressor, low expression associated with more aggressive subtype, poor survival	[98]
Osteosarcoma	NFIB	Tumour suppressor, SNP rs7034162 in NFIB significantly associated with lower NFIB expression, increased metastasis	[99]
Pilocytic Astrocytoma	NFIA	Oncogene, NFIA:RAF1 fusion activates the MAPK pathway	[74]
Small cell lung carcinoma	NFIB	Oncogene, increases chromatin accessibility, marks metastatic disease	[39, 65, 100]

Abbreviations

ADAM12	Disintegrin and Metalloprotease domain-containing protein 12
ATAC-seq	Assay for Transposase-Accessible Chromatin with high throughput sequencing
ChIP-seq	Chromatin immunoprecipitation sequencing
DNMT	DNA methyltransferase
ECM	Extracellular matrix
EZH2	Ehancer of Zeste Homolog 2
GR	Glucocorticoid Receptor
HDAC	Histone Deacetylase
LTR	Long Terminal Repeats
MeCP2	Methyl CpG binding protein 2
miRNA	micro RNA
MMTV	Mouse Mammary Tumour Virus
NFI	Nuclear Factor One
NFIA	Nuclear Factor One A
NFIB	Nuclear Factor One B
NFIC	Nuclear Factor One C
NFIX	Nuclear Factor One X
OCT1a	Octamer Factor 1
RNA-seq	RNA sequencing
SCLC	Small Cell Lung Cancer
SV40	Simian Virus 40