A Missing Hallmark of Cancer: Dysregulation of Differentiation

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Abstract

Cancer cells possess a nearly universal set of characteristics termed the hallmarks of cancer, including replicative immortality and resisting cell death. Dysregulated differentiation is present in virtually all cancers yet has not yet been described as a cancer hallmark. Like other hallmarks, dysregulated differentiation involves a breakdown of the cellular cooperation that typically makes multicellularity possible - in this case disrupting the division of labor among the cells of a body. At the time that the original hallmarks of cancer were described, it was not known that dysregulated differentiation was mechanistically distinct from growth inhibition, but now that this is known, it is a further reason to consider dysregulated differentiation a hallmark of cancer. Dysregulated differentiation also has clinical utility, as it forms the basis of pathological grading, predicts clinical outcomes, and is a viable target for therapies aimed at inducing differentiation. Here we argue that hallmarks of cancer should be near universal, mechanistically distinct, and have clinical utility for prognosis and/or therapy. Dysregulated differentiation meets all of these criteria.

Introduction

The identification of the hallmarks of cancer has been one of the most helpful and influential contributions to understanding cancer, because it brings simplicity, consistency and coherence to the otherwise overwhelming complexity of cancers\textsuperscript{1,2}. While cancer genomics has shown that each cancer is a unique mosaic of diverse genetic clones, evolutionary theory helps us
understand why this diversity often converges on strikingly similar phenotypes represented by the hallmarks\(^3\). We can view the hallmarks of cancer as the characteristics that are common across cancers, evolving consistently and independently in each cancer, because they confer a fitness benefit to the neoplastic cells over the surrounding normal somatic cells\(^3\). All complex multicellular organisms require cooperation between their individual somatic cells\(^4\).

Although complex multicellularity has evolved at least seven times\(^5\), there are five forms of cooperation upon which all multicellular organisms have converged upon: suppression of cell proliferation, controlled cell death, resource allocation, maintenance of the extracellular environment, and division of labor among the somatic cells\(^4\). Cancer, as a more general problem for multicellularity, can be understood as cells that cheat on the forms of cooperation necessary for building and maintaining a multicellular entitybody (Figure 1). All the current hallmarks of cancer map onto the five foundations of multicellularity, with one exception: there is no hallmark that corresponds to cheating on the division of labor among cells\(^4\). Here we suggest that there should be an additional hallmark of cancer which corresponds to this breakdown in division of labor. A breakdown of division of labor among cells would manifest as cells not adopting the proper cell types that are necessary for the proper functioning of the organism, i.e., dysregulated differentiation.
Figure 1. Cancer represents a breakdown of the foundations of multicellular cooperation that are necessary for multicellularity to succeed. The breakdown of every foundation of multicellularity corresponds to one or more of the existing hallmarks of cancer, with the exception of division of labor. Adding dysregulated differentiation as an additional hallmark of cancer fills this gap, corresponding to a breakdown in division of labor. There may well be other missing hallmarks, represented here as other gaps in the periphery.

Not only does dysregulated differentiation fill this gap, it also is already a well-recognized universal feature of cancer that is mechanistically distinct from other hallmarks, important for prognosis and a promising target for therapy. As we will argue in this perspective, the cancer
Hallmarks should not only be universal across cancers, but they should also be mechanistically distinct from one another, as well as diagnostically and therapeutically useful (Table 1). By these criteria, dysregulation of differentiation should be considered a hallmark of cancer.

| Cancer Hallmark                  | Mechanistically Distinct                                                                 | Diagnostically Functional                                                                 | Therapeutically Relevant                                                                 |
|----------------------------------|-----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| Sustained Proliferative Signaling| Constitutive activation of proliferative pathways[^6]                                    | Proliferative markers such as Ki-67 have been long used in staging/grading cancers[^7]   | Numerous compounds have demonstrated efficacy against known proliferative pathways[^8] |
| Evade Growth Suppressors         | Tumor suppressor pathways cannot be fully functional in metastatic disease[^9]          | Although characterized in childhood retinoblastoma, the RB pathway is mutated the majority of human cancers[^10] | RB mutation status can significantly guide the clinical management of a variety of cancer types[^10] |
| Avoid Immune Destruction         | Through the expression of self antigens and manipulation via the tumor microenvironment, many tumor cells escape immune destruction[^11] | Intratumor leukocyte infiltration can be used as a prognostic index determining anti-tumor immune activity[^12] | There are several therapeutic targets such as PD-1, PD-L1, CTLA4, and Th1 that can potentially counter immune evasion[^13,14] |
| Enable Replicative Immortality   | Cancer cells are able to restore and maintain telomere functionality[^15]               | Telomerase activity provides insight to tumor differentiation status[^16]                | Targeting cyclin dependent kinases such as PI3K could trigger cancer cell senescence[^17]. Telomerase is a target for cancer therapy[^18] |
Table 1. Every existing hallmark of cancer has the properties of being mechanistically distinct, diagnostically functional (providing information, either at the genetic, cellular, or tissue level that can be utilized by a physician in diagnosis or prognosis) and therapeutically relevant (providing identifiable targets for therapeutic intervention). In addition to being universal across cancers, dysregulated differentiation exhibits these three features as well, suggesting that it should be included as a hallmark of cancer.
A universal feature of cancer

Dysregulation of differentiation is a universal feature of cancers\textsuperscript{37,38}. Both genomic and histological evidence indicate that dysregulated differentiation is pervasive (Table 2). Cancers are generally diagnosed by histological features, detectable under a light microscope, that indicate that something has gone wrong in differentiation. Histological examination of differentiation status is a foundational method in the cancer grading system which has long been the cornerstone determining patient prognosis\textsuperscript{39,40}. These histological aberrations of differentiation are far ranging, including glands are that improperly formed or are missing altogether. Sometimes there is loss of regulation over a progenitor cell population, that has not fully differentiated, such that it expands to a pathological level, as occurs in most of the hematopoetic neoplasms\textsuperscript{37} as well as the undifferentiated clonal expansions in carcinomas. In fact, the generation of a new mass, a neoplasm, is probably impossible as long as differentiation is being properly regulated. Differentiation regulates the proper proportions and number of different cell types in every tissue. The epithelial-to-mesenchymal transition (EMT) common to many cancers is a further example of aberrant differentiation\textsuperscript{41,42}.

| Cancer Type | Genomic Evidence of Dysregulated Differentiation | Histological Evidence of Dysregulated Differentiation |
|-------------|-------------------------------------------------|--------------------------------------------------|
| Breast      | Down regulation of Gata-3 precludes healthy gland differentiation and disrupts luminal cell fate\textsuperscript{,43–46} | Tumor differentiation status defines grading scale and strongly predicts patient prognosis\textsuperscript{35,47,48} |
| Colorectal  | NDRG2 is expressed at low or undetectable levels in high risk/poor prognosis colorectal adenomas\textsuperscript{49,50} | Differentiation status of a tumor was more predictive of prognosis than invasive margin and DNA ploidy\textsuperscript{51} |
| Tissue       | Description                                                                 | Additional Information                                                                 |
|-------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| Prostate    | FOXA1 suppression in prostate carcinoma indicative of irregular differentiation patterns$^{52,53,54}$ | Lack of full differentiation in prostate cancer precludes the usefulness of serum prostate specific antigen in measuring tumor burden.$^{39,55}$ |
| Lung        | TRPC channel disruption signals stemcell-like differentiation status$^{56–58}$  | Differentiation status is an independent predictor of prognosis in non-small cell lung cancer$^{59,60}$ |
| Thyroid     | Suppression of Notch signaling mediated differentiation$^{61–63}$             | Diversity of thyroid carcinoma subtype founded largely on morphological differentiation$^{64–66}$ |
| Bladder     | Renewal of Hedgehog signaling pathway can illicit differentiation factors that improve prognosis$^{67,68}$ | Tumor cells reveal morphological indications of dysegulated differentiation before chromosomal aberrations$^{69,70}$ |
| Stomach     | Amplification of Notch1 intracellular domain maintains population of undifferentiated or poorly differentiated cells in carcinoma of the stomach$^{71–73}$ | Even well differentiated gastric carcinoma show histological evidence of disruption$^{74}$ |
| Cervical    | Expression of FOXC2 in cervical tissue correlates with increases in number of poorly differentiated cells$^{75,76}$ | Disruption of healthy differentiation can be detected with light microscope and/or positron emission tomography$^{77}$ |
| Non-hodgkin Lymphoma | Expression profiles show T-cell differentiation in B-NHL is skewed towards early stages$^{78}$ | Phenotypic classification of tumor cells by degree of differentiation informs prognosis$^{79,80}$ |
| Endometrial | Karyotypic aberration patterns correlate with histological differentiation$^{81}$ | Tissue specific differentiation and hormone receptor positivity are key prognostic factors$^{82–84}$ |
| Tissue                  | Comment                                                                 |
|------------------------|-------------------------------------------------------------------------|
| Leukemia               | Pax5 loss and t(15;17) translocations both cause differentiation blocks in leukemias<sup>85</sup> | A review of differentiation therapy for leukemia<sup>36,86</sup> Undifferentiated leukemia by light microscopy with myeloid features<sup>36,86,87</sup> |
| Kidney                 | Positive correlation between low PTEN expression and poorer differentiation<sup>88</sup> | Differentiation level by subtype predicts patient outcome<sup>89,90</sup> |
| Melanoma of the skin   | Melanoma differentiation associated gene-7 (MDA7) expression is downregulated in advanced melanoma and virtually undetectable in metastatic disease<sup>91</sup> | Differentiation status and like-ness with other skin markings provides a baseline understanding of disease state |
| Lip, oral cavity       | Absence of epithelial keratins defines a de-differentiated state in oral carcinomas<sup>92,93</sup> | Morphological differentiation status, although particularly subjective in the oral cavity, still associated with patient outcome<sup>94,95</sup> |
| Brain and Central Nervous System | Reactivation of Wnt signaling induce neural differentiation and cancer cell death 96–99 | Glioblastoma stem-like cells can hijack differentiation pathways to recruit vascularization<sup>100,101</sup> |
| Ovary                  | Notch1 overexpression increases with decreasing extent of fully differentiated cells<sup>102–105</sup> | Extent of morphologically poorly differentiated cells within ovarian tumor predicts prognosis<sup>82,106,107</sup> |
| Liver                  | MYC inactivation in an animal model of HCC induced differentiation and sustained regression of the tumor<sup>108</sup>. Increased LEF1 expression in hepatocellular cancer is associated with poor cellular differentiation and worse prognosis, and | Well differentiated hepatocellular carcinoma presents atypically and yet retains histological evidence of differentiation abrogations<sup>110,111</sup> |
| Tissue                  | Genetic and Histopathological Evidence |
|------------------------|----------------------------------------|
| Esophagus              | 22% of esophageal squamous cell carcinomas have mutations in genes that regulate esophageal squamous cell differentiation (NOTCH1, NOTCH2 or NOTCH3)\(^{112}\)  
In squamous cell carcinoma, Notch3 is repressed by TGF\(\beta\), which blocks terminal differentiation and leads to Notch1 mediated EMT\(^{113}\)  
Majority of esophageal carcinoma shows moderate to completely undifferentiated cell morphology\(^{114}\) |
| Larynx                 | Cyclin E overexpression in a majority of laryngeal carcinomas is a key driver of poorly differentiated tumors\(^{115}\).  
Lymphoepithelioma is an undifferentiated carcinoma of the nasopharyngeal type with propensity for metastasis\(^{116-118}\) |
| Multiple myeloma       | Maintained B cell expression of CD38 perpetuates a sub-differentiated population of cells clonally related to the multiple myeloma plasma cells\(^{119,120}\).  
Morphological indications of plasma cell differentiation level significantly predict clinical outcome\(^{121,122}\) |

**Table 2.** Evidence for disruption of differentiation, both genetic and histopathological, in the 20 most prevalent types of cancers worldwide\(^{123}\).

**Convergent somatic evolution**

Cells that stop devoting resources to the tasks inherent to that of their normal differentiated state, and instead devote those resources to proliferation and survival, will have a fitness advantage over cells that continue to devote resources to the specific tasks of their tissue type. 
Dysregulation of differentiation evolves independently in each cancer because it provides a selective advantage to those cells.
Differentiation is beneficial for organisms because it not only allows for the division of cellular labor, but also because it can lower cancer risk through reducing ongoing cell proliferation\textsuperscript{124}. This appears to be one of the mechanisms that organisms have evolved to prevent somatic mutations and the expansion of clones that acquire selective advantages from those mutations\textsuperscript{125}. In fact, there are many features of differentiated tissue architecture that function to constrain would-be clonal expansions. In intestinal crypts, which have a high rate of cell turnover, this function is performed by basal apical polarity axis maintained through a basement membrane attachment requirement, apical tight junctions between adjacent cells, and basal hemidesmosomal attachment complexes\textsuperscript{126–130}. Consequently, neoplastic cells gain a cell-level fitness advantage by evading those constraints\textsuperscript{3}. The suspension of proliferative abilities in fully differentiated cells is one of the major mechanisms of somatic-level evolutionary suppression, in other words it is a cancer resistance mechanism. However, this also means that there is strong selective pressure on neoplastic cells to evolve the ability to evade full differentiation.

Cairns first pointed out in 1975 that if mutations are gained in transit amplifying cells, which are only partially differentiated, these cells will quickly be flushed from the body with little chance to accumulate additional mutations necessary to cause cancer\textsuperscript{125} (Figure 2). In this way, differentiation in tissues with high cell turnover acts as a tumor suppressor. Follow-up mathematical and computational models have shown that alterations in differentiation are likely some of the most universal early lesions in neoplastic progression\textsuperscript{131,132}. 
In order for a neoplasm to grow, cells must somehow evade the inexorable conveyor belt of differentiation that transforms stem cells into progressive stages of transit amplifying cells, eventually becoming fully differentiated cells, and finally exiting the tissue by apoptosis. There are two ways a neoplasm may form: 

**a.)** A clone of stem cells may stop producing transit amplifying cells, only dividing symmetrically to produce daughter stem cells. That clone will have a competitive advantage over any stem cell clones that continue to use some of their resources to produce transit amplifying cells. This may be due to an abrogation in the clone’s differentiation pathways or through the gaining of independence from stem cell niche signals that would otherwise be required to maintain the stem cell state.

**b.)** Alternatively, transit amplifying cells may stop differentiation and so effectively step off of the conveyor belt of differentiation. This gives the non-differentiating (and thus self-renewing) transit amplifying cells a competitive advantage over transit amplifying cells that continue to differentiate.

Both stem cells and transit amplifying cells gain fitness advantages from disrupting differentiation. However, there are generally many more transit amplifying cells than stem...
cells and so some mathematical models predict that most cancers derive from transit amplifying cells, even if that requires additional mutations to disrupt differentiation\textsuperscript{132}. In order to become cancerous, transit amplifying cells must avoid the fate of being sloughed from the off of a proliferating tissue (Figure 2b). Any stem cell that disrupts differentiation and divides symmetrically, producing two daughter stem cells, will have a fitness advantage over other stem cells that divide asymmetrically and use some of their resources to produce non-stem cells (Figure 2a). In summary, there are good evolutionary reasons to expect that virtually all cancer cells can gain a fitness benefit from disrupting differentiation, which explains why dysregulation of differentiation consistently evolves and is a universal feature of cancers.

**Mechanisms of dysregulation**

In their original hallmarks paper, Hanahan and Weinberg discuss the apparent strategy of tumor cells to promote growth by avoiding terminal differentiation. The authors specifically cite the Mad-Max complex, the inactivation of APC/B-catenin pathway in colon carcinogenesis, and the erbA oncogene in avian erythroblastosis\textsuperscript{2}. At the time, the dysregulation of differentiation was not included in the hallmarks of cancer because the mechanisms of differentiation could not be distinguished from an insensitivity to antigrowth signals and limitless replicative potential. It was not clear whether loss of differentiation was simply a loss of growth inhibition or an independent factor in carcinogenesis.

In general, differentiation and growth inhibition are tightly, and mechanistically coordinated. However, there are instructive cases of fully differentiated cells that are still proliferative, including beta cells in the pancreas, hepatocytes in the liver, T-cells, and fibroblasts in numerous tissue types\textsuperscript{134–140}. These exceptions show that there is a fundamental distinction between loss of proliferative ability and differentiation, though they co-occur often.

There has been ample documentation of interruptions in key differentiation pathways, separate from growth inhibition pathways, that are conserved across cancer types, ultimately preventing true terminal differentiation. Notch signaling plays a complex, and not fully understood, role in distinct differentiation signaling pathways\textsuperscript{141}. In T-cell acute lymphoblastic leukemia (T-ALL) chromosomal translocations result in constitutive Notch1 signaling that precludes terminal
Rangarajan and colleagues demonstrated that Notch1 deletion in keratinocytes resulted in hyperplasia and generalized dysregulation of known differentiation markers. In addition, constitutive expression of the MYC oncogene is common in human cancers and has a well-established role in prevention of differentiation and sustaining proliferative signals. For instance, in a murine model of liver cancer inactivation of MYC was sufficient to differentiate the tumor into normal hepatocytes. Interestingly, c-myc expression drives the differentiation of keratinocytes where Notch1 appears to play the role of a tumor suppressor. The identification of differentiation specific pathway alterations holds the potential to serve as an indicator for therapeutic response. In the crypt structures of the intestinal epithelium, progenitor stem cells are characterized by high expression of Leucine-rich repeat-containing G-protein coupled receptor (LGR5). Similarly high levels of expression are seen in colorectal cancers, where it is indicative of catastrophic Wnt/β-catenin signaling deregulation and worse patient outcomes.

**Well-differentiated tumors**

Well-differentiated cancers sometimes can be difficult to distinguish from reactive changes in tissue, such as hyperplasia, or benign tumors by light microscopy. However, even if indiscernible by visual inspection, molecular evidence shows the presence of dysregulated differentiation in well-differentiated tumors. Well-differentiated tumors exhibit a less differentiated molecular profile with elevated expression of precursor genes and lower expression of tissue specific genes compared to healthy tissue. Gene expression studies in histologically differentiated thyroid cancers have found a disruption in differentiation on a molecular level as compared to benign thyroid tissue. Using primary thyroid cancers, Yu et al demonstrated that Notch-1 expression was downregulated in differentiated thyroid cancer tissues compared to benign thyroid tissues and that decreased Notch-1 expression was associated with more aggressive tumors with extrathyroidal invasion. Restoration of Notch-1 expression in a metastatic, differentiated thyroid carcinoma cell line led to a reduction in cell growth and tumor cell migration. The Cancer Genome Atlas Research Network investigated the relationship between driver mutations BRAFV600E and RAS and differentiation in papillary thyroid cancer,
a typically well-differentiated cancer by histology\textsuperscript{167}. Differentiation of over 350 PTCs was quantified and scored by measuring mRNA expression of 16 thyroid function genes, with a lower score indicating decreased differentiation\textsuperscript{167}. Interestingly, increased differentiation scores were correlated with the PTC driver mutation BRAF\textsuperscript{V600E}, while decreased differentiation scores were correlated with the driver mutation RAS\textsuperscript{167}. Further, upon pathological examination, tumors with lower differentiation scores by mRNA expression were found to have subtle architectural changes that generated more poorly formed and complex papillary structure with fewer follicles\textsuperscript{167}. These findings suggested that certain driver mutations may contribute to decreased differentiation in thyroid cancer\textsuperscript{167}. As the new molecular tools under development are advanced for clinical application, the feasibility of identifying lack of terminal differentiation even in the most well-differentiated cancers increases.

**Prognostic importance**

Poorly differentiated cancer cells are known to be much more aggressive than their well differentiated counterparts, a fact which plays a critical role in predicting patient outcome\textsuperscript{39,74,168,169}. Well-trained pathologists have long been able to accurately assign patient prognosis through tumor grade although the process is heavily burdened with the inherent subjectivity in assessing differentiation optically and the morphological variation that is inherent in most tumors. Advances in cancer genomics have validated genetic attributes that resemble stem cells\textsuperscript{170–172} as prognostic indicators. Similar to histopathological grading systems, differentiation gene-expression profiles can predict patient outcomes\textsuperscript{170–172}. A 2017 study examined the global gene expression profile of cancer cells and stratified them based on their distance in expression from that of stem cells to fully differentiated cells, using several different histologies including carcinomas, sarcomas, and hematologic malignancies\textsuperscript{173}. This methodology allowed for the derivation of a novel cancer gene expression signature found in all undifferentiated forms of the diverse cancers studied. For all subtypes analyzed, tumors most similar in expression to stem cells were both histologically less differentiated and clinically more aggressive. Furthermore, they also demonstrated that where a cancer fell on this spectrum predicted the patient’s survival. Work by Riester et al.\textsuperscript{173} and others has shown that there are objective measures of cellular differentiation, utilizing descriptive genetic profiles that detail where on a spectrum from “stemness” to full differentiation a given cancer cell lies. Grading
with molecular assays that measure the hallmarks of cancer enriches our ability to make clinical predictions while introducing novel quantification of differentiation status through genetic analysis.

Promising clinical opportunities
Differentiation should be considered a hallmark of cancer not only due to its universality and distinct cellular mechanisms that drive cancer, but also because the biological mechanisms can be targeted by available therapies that have already shown promise in the clinic. Rather than killing both healthy and tumor cells as do typical chemotherapeutics, differentiation therapies capitalize on the ability of cytokines to promote terminal differentiation of tumor cells and halt their capacity to self-renew\textsuperscript{174–177}. This option is especially promising for patients suffering from comorbidities who are unable to receive high-dose chemotherapy due to its significant toxicity.

The first successful clinical application of differentiation therapy was the use of All-trans Retinoic Acid (ATRA) for acute promyelocytic leukemia (APL). ATRA induces APL blasts to terminally differentiate\textsuperscript{36}. The current standard of care for treatment of APL involves the combination of ATRA and arsenic, making APL now a highly curable disease with 5-year disease-free survival rates that exceed 90\%\textsuperscript{178}.

Outside of APL, differentiation therapy has been gaining traction in the treatment of acute myeloid leukemia (AML)\textsuperscript{36,179–182}. A recent preclinical study has identified a novel, highly potent and selective inhibitor that induces differentiation \textit{in vitro} and \textit{in vivo} by inhibiting dihydroorotate dehydrogenase across multiple AML subtypes\textsuperscript{179}. A promising phase-1 trial of this inhibitor, BAY 2402234, is currently ongoing for myeloid malignancies (NCT 03404726).

Whether differentiation therapy shows similar effects in cancers apart from the hematological malignancies is worth investigating. Cancer stem cells (CSCs), also known as tumor-initiating cells, represent one such target for differentiation therapy\textsuperscript{183–189}. First identified in AML in 1997, they have since been identified in brain cancer, colon cancer, pancreatic cancer, prostate cancer, melanoma, and more\textsuperscript{190,191}. They are highly resistant to traditional chemotherapy and radiotherapy, which may be due in part to their relative slow growth and high expression of
anti-apoptotic proteins\textsuperscript{\hspace{1em}192}. Differentiation therapy is a promising tactic that may induce these CSCs into non-stem cancer cells with limited self-renewal potential. These non-stem cells could possibly be better targeted by conventional therapies\textsuperscript{\hspace{1em}193,194}.

Another logical application of differentiation therapy would be in tumors that are collectively known as “blastomas” or small round blue cell tumors, named after their histological appearance, which is monotonous and characterized by lack of differentiation features. These relatively undifferentiated tumors originate from stem cell progenitors and occur almost exclusively in pediatric patients. Neuroblastoma is one of the small round blue cells tumors. It is famous for frequent spontaneous regression or differentiation into a benign ganglioneuroma\textsuperscript{\hspace{1em}195}. Recent evidence shows that neuroblastomas are composed of cells from two super-enhancer associated differentiation states: undifferentiated mesenchymal cells and committed adrenergic cells\textsuperscript{\hspace{1em}196}. Nevertheless, cells from either state can interconvert, highlighting a potential mechanism of tumor relapse as mesenchymal cells are known to be relatively resistant to chemotherapy\textsuperscript{\hspace{1em}196}. Furthermore, these preserved differentiation pathways have been successfully targeted in vitro with retinoids\textsuperscript{\hspace{1em}197,198}, a response categorized with cell proliferation arrest and a markedly lower MYCN expression\textsuperscript{\hspace{1em}197}.

Differentiation therapy can only work if some differentiation pathways remain intact in a cancer and can be stimulated by an intervention. Due to natural selection at the somatic level for the dysregulation of differentiation, it may not always be possible to induce differentiation.

**Conclusions**

Dysregulation of differentiation is a universal phenotype, found in virtually all cancers (Table 2). The degree of differentiation has long been used in oncology for diagnosis as well as prognosis, and advances in genomic analyses have shown promise for improving prognosis. Dysregulation of differentiation is molecularly distinct from the other hallmarks, including evading growth suppressors, and it has been successfully targeted for therapy in acute promyelocytic leukemia and neuroblastoma. Further, it is clear that dysregulated differentiation is a breakdown of multicellular cooperation, and the only aspect of this breakdown of multicellular cooperation that is not already represented in the hallmarks of cancer\textsuperscript{\hspace{1em}4}. Together, this suggests that dysregulated
differentiation is a missing hallmark that should be added to the commonly accepted list of shared phenotypes of cancer.

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