

# Evolving transcriptomic fingerprint based on genome-wide data as prognostic tools in prostate cancer

Mohammed Alshalalfa, Mark Schliekelman, Heesun Shin, Nicholas Erho and Elai Davicioni

GenomeDx Biosciences, Vancouver, BC, Canada

**Background Information.** Prostate cancer (PCa) is a common disease but only a small subset of patients are at risk of developing metastasis and lethal disease, and identifying which patients will progress is challenging because of the heterogeneity underlying tumour progression. Understanding this heterogeneity at the molecular level and the resulting clinical impact is a critical step necessary for risk stratification. Defining genomic fingerprint elucidates molecular variation and may improve PCa risk stratification, providing more accurate prognostic information of tumour aggressiveness (or lethality) for prognostic biomarker development. Therefore, we explored transcriptomic differences between patients with indolent disease outcome and patients who developed metastasis post-radical prostatectomy using genome-wide expression data in the post radical prostatectomy clinical space before metastatic spread.

**Results.** Based on differential expression analysis, patients with adverse pathological findings who are at higher risk of developing metastasis have a distinct transcriptomic fingerprint that can be detected on surgically removed prostate specimens several years before metastasis detection. Nearly half of the transcriptomic fingerprint features were non-coding RNA highlighting their pivotal role in PCa progression. Protein-coding RNA features in the fingerprint are involved in multiple pathways including cell cycle, chromosome structure maintenance and cytoskeleton organisation. The metastatic transcriptomic fingerprint was determined in independent cohorts verifying the association between the fingerprint and metastatic patients. Further, the fingerprint was confirmed in metastasis lesions demonstrating that the fingerprint represents early metastatic transcriptomic changes, suggesting its utility as a prognostic tool to predict metastasis and provide clinical value in the early radical prostatectomy setting.

**Conclusions.** Here, we show that transcriptomic patterns of metastatic PCa exist that can be detected early after radical prostatectomy. This metastatic fingerprint has potential prognostic ability that can impact PCa treatment management potentially circumventing the requirements for unnecessary therapies.



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## Introduction

Prostate cancer (PCa) is a molecularly and clinically heterogeneous cancer which renders its progression unpredictable (Nwosu *et al.*, 2001; Boyd *et al.*, 2012; Wyatt *et al.*, 2013). PCas that appear clinically similar at surgery often exhibit a range of clinical

outcomes that is not captured by existing risk categories (*e.g.* D'Amico criteria) or prognostic tools such as prostate-specific antigen (PSA), Gleason grade or tumour stage (Erho *et al.*, 2013). Although these tools continue to be important in risk assessment, they lack the precision required to guide decisions regarding treatment options. Nearly 50% of patients treated with surgery will have adverse pathology or

To whom correspondence should be addressed to (email: mohamed@genomedx.com)

**Key words:** Metastasis, Prostate, Radical prostatectomy, Transcriptomic fingerprint.

**Abbreviations:** BCR, biochemical recurrence; MFD, median fold difference; PCa, prostate cancer; PSA, prostate-specific antigen; RP, radical prostatectomy; RF, random forest.

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subsequently develop a PSA rise and are therefore considered at increased risk for developing clinical recurrence (Nakagawa *et al.*, 2008). However, according to Boorjian *et al.* (2011), 12% of patients who developed biochemical recurrence (BCR) after radical prostatectomy (RP), presented a systematic progression at certain point. Patients with PSA rise post-surgery and no metastasis for at least 10 years were shown to be no different molecularly than patients with no evidence of developing PSA rise (Alshalalfa *et al.*, 2015). Incorporating genomic data effectively stratified patients at risk of developing metastasis post PSA rise (Ross *et al.*, 2014). Recently, the incorporation of genomic data with clinical risk assessment has shown potential to improve risk stratification at all stages of disease (Cooperberg *et al.*, 2015).

The genomic alterations underlying the transition of PCa from an indolent state to a more aggressive nature have not been fully explored. With advances in high-throughput technology, our understanding of the genomic changes responsible for the development and progression of PCa has expanded (Taylor *et al.*, 2010; Berger *et al.*, 2011; Barbieri *et al.*, 2013). Large scale 'omics' approaches such as microarray and next-gene sequencing have generated a large body of data that has enormously increased our knowledge of the functional role molecular alterations play in cancer progression and offers an opportunity to integrate molecular data to provide improved risk assessment (Erho *et al.*, 2013). Genomics-informed cancer medicine offers the potential for precision cancer treatment by delivering individualised information in the clinic that providers and patients can use to make personalised medical decisions. As an example, genomic fingerprinting may impact clinical decisions for localised PCa by providing individualised cancer progression risk estimates of disease recurrence after surgery that can inform at diagnostic or post-operative treatment decisions (Davis, 2014). Likewise, several multi-gene fingerprints have expanded the landscape of the breast cancer diagnostics (Ross *et al.*, 2008).

Large-scale omics approaches have enabled the discovery and development of diagnostic and prognostic biomarkers for personalised medicine (Yuan *et al.*, 2014). An increasing number of biomarkers (Figure 1A) discovered at various stages of disease progression contribute to the early diagnosis of PCa

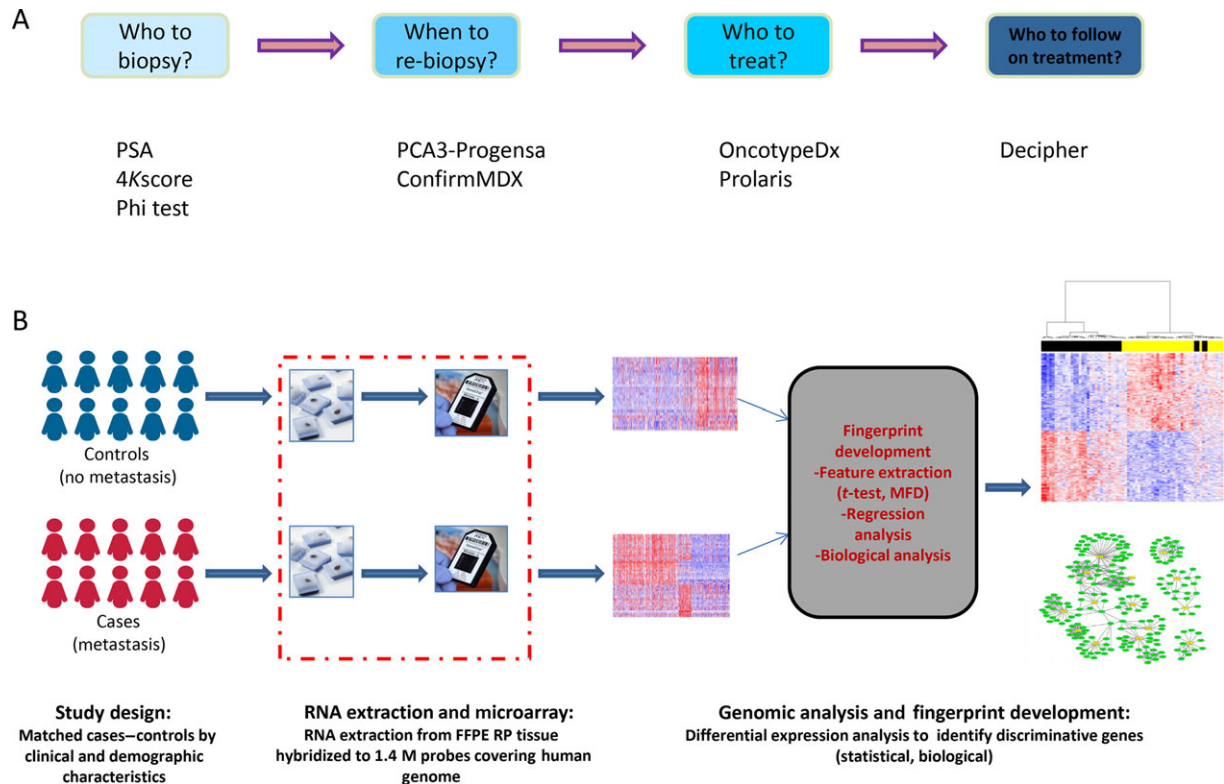
(Leyten *et al.*, 2014), predict BCR (Taylor *et al.*, 2010) and identify high-risk patients that may avoid unnecessary treatment (Karnes *et al.*, 2013). Among the most common molecular events in early prostate tumour development is the rise of PSA (Tosoian and Loeb, 2010; Payne and Cornford, 2011; Prensner *et al.*, 2012). Although highly sensitive, PSA measurement provides poor specificity for clinically significant disease detection and can result in unnecessary biopsies leading to over-diagnosis and over-treatment of indolent tumours that might be managed more conservatively. Currently, two sets of biomarkers have improved risk stratification on follow-up biopsy: prostate cancer antigen 3 (*PCA3*) and methylation level of *GSTP1*, *APC* and *RASSF1*. *PCA3* is commercially available as Progenesa and *GSTP1*, *APC* and *RASSF1* methylation as ConfirMDx. The *PCA3* mRNA level in urine is positively associated with cancer detection and better able to predict the presence of cancer on repeat biopsy than PSA alone (Hessels and Schalken, 2009; Durand *et al.*, 2011). ConfirMDx utilises the methylation pattern of these three genes to identify men at low risk on follow-up biopsy (Trock *et al.*, 2012). Other single gene diagnostic biomarkers like *AMACR* (from tissue) (Jiang *et al.*, 2013) and *TMPRSS2-ERG* gene fusions (in urine) were demonstrated to be specific PCa biomarkers (Tomlins *et al.*, 2011). Multiple studies improved early detection of PCa by combined these biomarkers (Perdonà *et al.*, 2013; Salami *et al.*, 2013; Leyten *et al.*, 2014).

At a positive cancer diagnosis, two commercially available gene panels (Cuzick *et al.*, 2012; Klein *et al.*, 2014) have been developed to discriminate between clinically indolent and potentially high-risk patients to avoid overtreatment. Other gene panels such as the Penny gene signature (Penney *et al.*, 2011) was developed using a gene array (~6000 genes) capturing the difference between low and high Gleason scores and then evaluate it in intermediate Gleason score patients. Other gene panels (Markert *et al.*, 2011; Irshad *et al.*, 2013; Jin *et al.*, 2014) were discovered and validated on single institute data sets and claim to be associated with lethal PCa, although only a few have made it into the clinic.

Gene expression profiling of tumour samples from RP has the capacity to characterise the tumour biology and help identify patients who may develop metastasis several years after RP (Erho *et al.*,

**Figure 1 |**

(A) Prostate cancer diagnostic and prognostic tools are commercially available at various stages of cancer. (B) A flow diagram of developing genome-wide prognostic tools. It starts with the relevant clinical question, designing the study, using the most informative technology and applying the appropriate feature filtering methods.



2013). Identifying post-RP patients with adverse pathology that can be safely monitored without further intervention and patients who should be put on a second line of treatment is a significant and unmet need. Characterising the biology underlying tumour aggressiveness is of paramount importance to characterise tumour outcome and develop biomarkers that offer prognostic information with the expectation of better post-surgical treatment. This was the rationale for the development of the Decipher PCa genomic test to identify patients who will develop metastasis within 5 years after surgery (Erho *et al.*, 2013).

In this work, we delineate the molecular biology underlying metastasis after RP to identify a transcriptomic fingerprint of metastatic PCa and then, using the Decipher pipeline as an example, go through a series of steps to demonstrate delivery of the fingerprint into the clinic as prognostic tool.

## Results and discussion

### Clinical characteristics of the study cohort

In order to develop and evaluate a metastatic transcriptomic fingerprint, 1449 RP patients were pooled from eight studies Table S1 that had samples profiled on Affymetrix Human Exon 1.0 ST arrays. The discovery cohort, a case-control design, (Mayo Clinic I) is an intermediate-high risk cohort of European Americans; 70% developed BCR and 40% developed clinical metastasis Table 1. BCR was defined as two successive increases in PSA measurement above 0.2 ng/ml with subsequent measure of 0.05 ng/ml above the first measurement. Clinical metastasis was confirmed by bone or CT scan. Seventeen percent developed metastasis within 5 years with a median follow up of 41 months, and 18% developed metastasis between 5 and 10 years with median follow up of 88 months. The eight cohorts used for this study represent the spectrum of PCa treated with radical

**Table 1 | Clinicopathological characteristics of the patients used for discovering and evaluating the metastatic fingerprint**

	Discovery	Evaluation
Race	545	904
European American	545(100%)	734(81.2%)
African American	0	57(6.31%)
Others	0	10(1.11%)
NA	0	103(11.4%)
Patient age (yr)		
Median [range]	66 [47, 79]	61 [37.3, 83]
Pre-operative PSA		
Pre-PSA <10	286(51.91%)	371(41.04%)
10 ≤ pre-PSA <20	118(21.42%)	158(17.48%)
Pre-PSA >20	132(23.96%)	86(9.51%)
NA	15(2.72%)	289(31.97%)
Path GS		
<=6	64(11.62%)	170(18.81%)
7	275(49.91%)	521(57.63%)
8	68(12.34%)	100(11.06%)
>=9	144(26.13%)	110(12.17%)
NA	0(0%)	3(0.33%)
EPE		
Positive	276(50.09%)	378(41.81%)
NA	0(0%)	242(26.77%)
SVI		
Positive	177(32.12%)	173(19.14%)
NA	0(0%)	239(26.44%)
SM		
Positive	270(49%)	364(40.27%)
NA	0(0%)	99(10.95%)
LNI		
Positive	73(13.25%)	60(6.64%)
NA	0(0%)	79(8.74%)
Met		
Positive	215(39.02%)	148(16.37%)
NA	0(0%)	191(21.13%)
BCR		
Positive	393(71.32%)	275(30.42%)
NA	0(0%)	191(21.13%)

Overall, about 61% of patients manifest with adverse pathologic findings, and patients are mostly of European descent.

prostatectomy from low to high-risk localised disease. Overall, 61% of patients in the pooled cohort had one or more adverse pathology findings ranging from 5% to 89%, and 22% (on average) of patients developed metastasis ranging from 7% to 40% Table 1.

### Development of transcriptomic fingerprint of metastasis

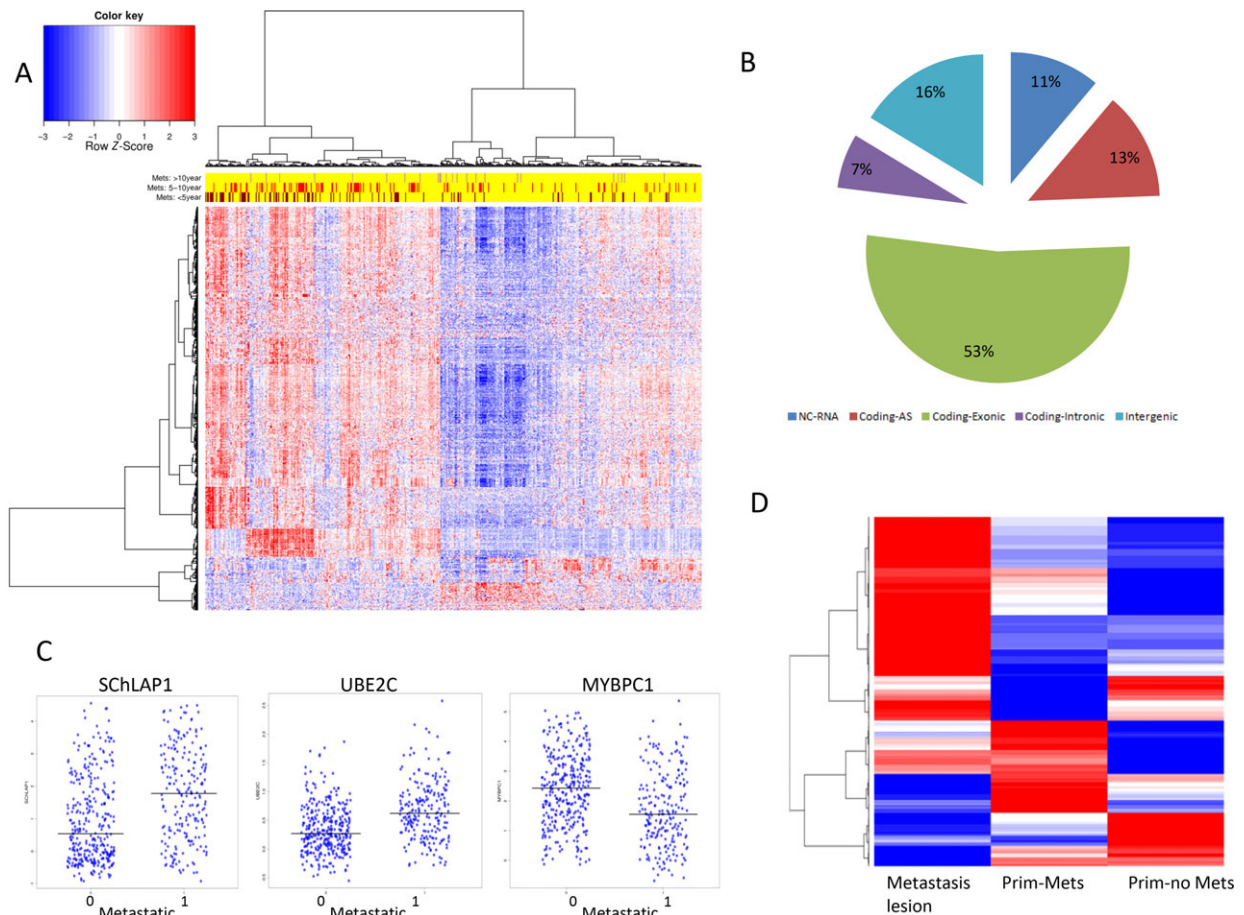
In this study, we sought to determine whether a genomic fingerprint of metastasis could be detected in the primary tumours of patients who develop metastatic disease through comparison with primary

tumours of patients that do not develop evidence of metastasis even after long clinical follow up. First, we divided the Mayo Cohort I into metastatic (cases) and non-metastatic (controls) and applied discriminative analysis to filter informative features (Figure 1B). Differential expression analysis identified 568 up-regulated and 86 down-regulated probesets in patients that developed metastasis (metastatic group), suggesting there is a transcriptomic fingerprint identifying patients at higher risk of developing metastasis after RP, long before the clinical detection of metastasis (Figure 2A). We investigated the representation of protein coding and non-coding regions of the genome in the fingerprint detected in this analysis (metastatic fingerprint); approximately half of the 654 features in the fingerprint are non-coding RNA including long non-codingRNAs (lncRNAs) (11%), intergenic (16%) and anti-sense probsets (13%) (Figure 2B; Table S2). lncRNAs including the *SCbLAP1* region, and *NEAT1* were among the most up-regulated features in the metastatic fingerprint (Figure 2C). On their own, *SCbLAP1* and *NEAT1* have been demonstrated to be independent predictors of metastasis in two separate studies (Chakravarty *et al.*, 2014; Prensner *et al.*, 2014). Here, we confirmed those findings and add to the growing body of evidence supporting a key role for long non-coding RNA in PCa disease progression. We found the lncRNA *PCAT18* is down-regulated in metastatic patients, although other studies have shown it to be subsequently up-regulated in metastatic castration-resistant disease (Crea *et al.*, 2014), suggesting that *PCAT18* may play a role in developing castration resistance. Several reports show that these lncRNAs are prostate specific, suggesting that they have specific role in PCa progression (Martens-Uzunova *et al.*, 2014). Delineating the functional role of these lncRNAs requires further investigation that is not within the scope of this investigation.

To gain more biological insights into the metastatic fingerprint, Gene Enrichment Analysis using DAVID web services (Dennis *et al.*, 2003) showed that up-regulated genes are highly enriched with cell cycle processes ( $P = 8E-9$ ), cytoskeleton organisation ( $P = 2E-3$ ) and ECM-receptor interaction ( $P = 1.3E-3$ ), biological processes that are all involved in metastasis down-regulated genes were associated with protein kinase cascade ( $P = 3.2E-3$ ) and signal transduction ( $P = 0.02$ ). The top

**Figure 2 | Metastatic fingerprint ( $n = 654$ ) discovered from the Mayo Clinic I showing that most of the features are up-regulated in the metastatic group**

(A) Heatmap showing three main correlated groups of upregulated genes, among which is the *SChLAP1* region. (B) Approximately half of the features are non-coding (lncRNA, intronic, intergenic and anti-sense). (C) *SChLAP1* and *UBE2C* are the top upregulated lncRNA and coding RNA respectively in the metastatic group, and *MYBPC1* is the most down-regulated. (D) The metastatic fingerprint is representing the biology of metastasis tissues from MSKCC. Most of features up-regulated in localised cancer with metastatic outcome (Prim-Met) are also up-regulated in metastasis lesions.



down-regulated genes were Myosin-Binding Protein C (*MYBPC1*) (Figure 2C) and Anoctamin7 (*ANO7*); the first plays a structural role to preserve cell rigidity and the second maintains cell-cell interactions. On the other hand, the top up-regulated genes were *UBE2C*, *TOP2A*, *CAMK2N1*, *CENPF* and *ZWILCH*. All of these genes are involved in cell cycle processes, mitotic spindle assembly and mitotic checkpoints, and have been previously reported to be associated with PCa recurrence (Cuzick et al. 2012) and castration-resistant PCa (Chen *et al.*, 2011). Likely, overexpression of these genes allows

the cell to proliferate, re-organise its skeleton and escape cell cycle control mechanisms thereby avoiding pro-apoptotic signals.

### The metastatic fingerprint in metastatic tissues

Further confirming that the metastatic fingerprint from the primary tumour represents transcriptomic changes that are also present in the metastatic lesions, we compared the expression data of the metastatic fingerprint from 19 metastatic lesions and 131 primary PCa as a control set from the MSKCC cohort (9 with metastatic outcome) (Figure 2D). We found that

30% of the metastatic fingerprint is overexpressed in the 19 metastatic lesions samples including *TOP2A*, *TMSB10*, *SCbLAP1*, *STMN1* and *MKI67* confirming that the metastatic fingerprint represents the early stages of metastasis development. Functional analysis of these features showed that they are mainly involved in cell cycle, cytoskeleton protein binding and ECM–receptor interaction. We observed that not all the features contributed equally to distinguishing patients with metastasis cancer from patients with localised cancer (Figure 2D). Interestingly we found that *NEAT1* expression did not change in the metastatic lesions patients, suggesting an early role of *NEAT1* to drive metastasis. Approximately 10% of the genes were further down-regulated in metastatic lesions, including *MYBPC1*, *ANO7* and *AZGP1*, compared with primaries with metastasis outcome. These genes were mainly enriched for functions in focal adhesion. Approximately 10% of the features were down-regulated in the metastasis lesions after being relatively overexpressed in the primary tumour with metastatic outcome including *SFRP4*, *ASPN* and *GNPTAB*. These genes enriched with ECM–receptor interaction, small cell lung cancer genes and glycosaminoglycan binding.

These results suggest that most of the features of the metastatic fingerprint play roles throughout the metastasis development process with only a small portion playing a role in early stages to drive metastasis.

This could indicate that the primary tumour contains cells with high metastatic potential that may have already spread to lymphatic system or bone; perhaps in a dormant state, until they are unmasked or revealed during the aging process. An alternative hypothesis could be that primary tumour has gained the early genomic changes needed for spreading and are in a transition state waiting upon endogenous or external signals to spread out, a model similar to that proposed in breast cancer metastasis (Weigelt *et al.*, 2005)

### Evaluating the metastatic fingerprint in RP cohorts

The most challenging and critical step for a fingerprint to be clinically adopted is validating the model in independent cohorts to show equivalent performance and demonstrate clinical impact on decision making (Figure 3A). To further verify the relevance of the metastatic fingerprint to metastatic patients, we evaluated the fingerprint in two independent datasets

(Figure 3B) from Mayo Clinic and CCF. Most of the features of the fingerprint were prognostic in the validation cohorts (Table S2). Hierarchical clustering of the patients in each cohort using Pearson's correlation and Ward's method aggregated most metastatic patients together, verifying the associations between the fingerprint and metastasis. Patients in the two evaluation sets were subtyped into ERG<sup>+</sup>, ETS<sup>+</sup>, SPINK<sup>+</sup> and TripleNeg groups to assess if the metastatic fingerprint is associated with certain subtype. We found the clustering of high risk patients to be independent of these subgroups (Figure 3B), demonstrating equivalent performance across different subtypes. Efficacy across common genetic subtypes is required for genomic tests to be part of standard clinical practice. For instance, the discrimination power of the Decipher test was found to be independent of the molecular subtypes (Figure 3C), suggesting that Decipher is robust in a heterogeneous population.

### Translating fingerprint into prognostic tools:

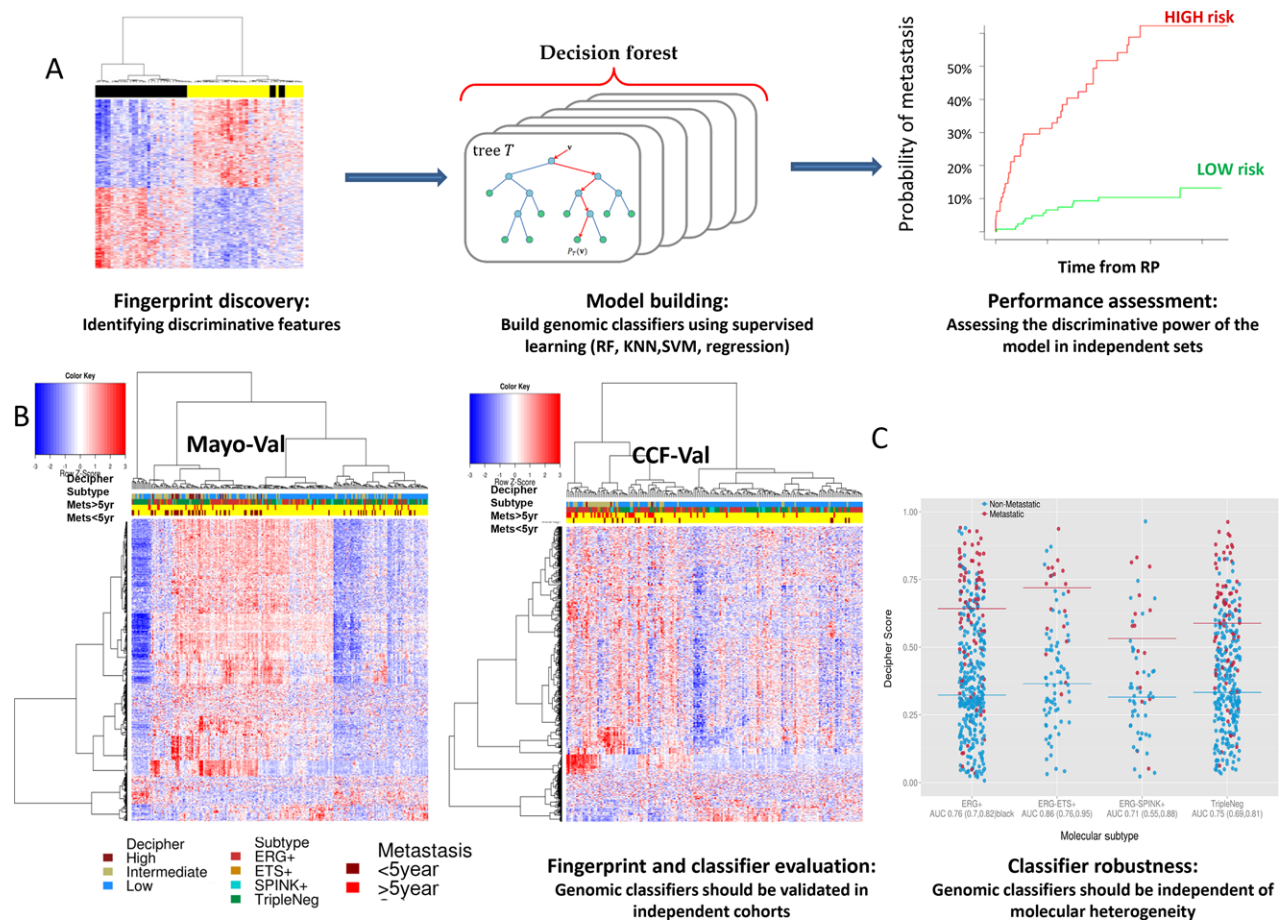
#### The Decipher PCa classifier

Delivering a fingerprint into the clinic as a diagnostic or prognostic tool starts with asking the relevant question, appropriate study design, using the appropriate methodologies, validating it the right setting resulting in clinical benefit. We first designed the study to define a fingerprint from the discovery cohort and then evaluated it in independent cohorts using clustering analysis. As clustering is not an appropriate approach to be used to predict prognosis of each new patient, more rigid models such as weighted regression or random forest (RF) modelling are used to generate scores that are easier to apply cut-points or categorise into risk groups based on an algorithm. In this case, we desired to show the relevance of the fingerprint to the metastatic group in independent cohorts. To provide a relevant example of translating a fingerprint into a prognostic tool, we use the Decipher classifier to demonstrate transitioning from fingerprint to clinical use, starting from transcriptomic fingerprint discovery to commercially available prognostic test.

Decipher is a transcriptomic fingerprint for metastasis that has been developed as a genomic prognostic test to predict the probability of metastasis after RP. Twenty-two RNA biomarkers filtered from 1.4 M probesets covering most of the human genome constitute the Decipher test. Several filtering criteria including Lasso regression were adopted to reduce

**Figure 3 | Delivering a fingerprint into the clinic requires appropriate modelling and effective performance in independent cohorts to validate the prognosis ability**

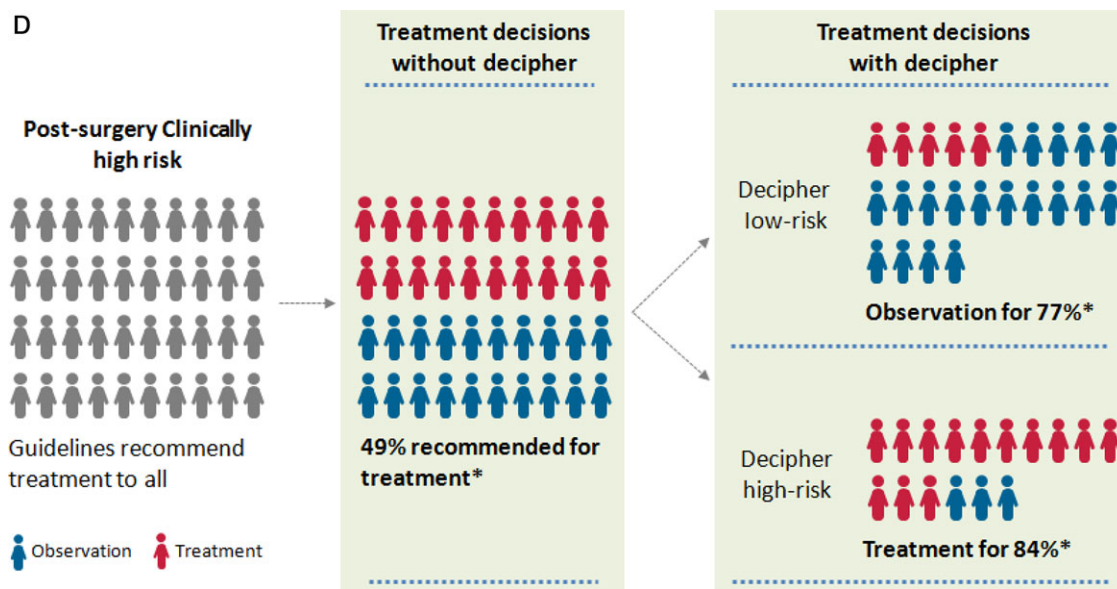
(A) Modelling fingerprint to genomic classifiers is a critical step to show utility of the fingerprint. It requires external independent cohorts to assess its performance. (B) The metastatic fingerprint from this work is showing high relevance to metastatic groups in two independent cohorts, and to Decipher risk categories. Metastatic patients fall into ERG<sup>+</sup>, ETS<sup>+</sup>, SPINK<sup>+</sup> and TripleNeg subgroups (annotation bar) suggesting a molecular heterogeneity underlying metastasis. (C) Decipher was shown to be independent of molecular subtypes and effective prognostic tool in all subtypes. (D) Ultimate goal of each prognostic tool is to have an impact on treatment decision making. Decipher more appropriately directs post-RP treatment to patients most at risk of metastasis.



the discriminative features into the most informative, non-redundant features, resulting in the 22 RNA biomarkers that best discriminated patients who developed metastasis within 5 years from patients that did not develop metastasis. These 22 RNA biomarker were modelled into a RF model that generates a score between 0 and 1; the higher the value the more likely the patients will develop metastasis. The model was validated in several cohorts and surpassed currently used clinical tools demonstrating

clinical utility (Karnes *et al.*, 2013; Den *et al.*, 2014; Klein *et al.*, 2014b). Combining the fingerprint genes into a single model with subsequent independent validation are the most critical in translating the fingerprint into a clinically meaningful score. Modelling the fingerprint requires a clean set of training data to maximise the discrimination power of the fingerprint features collectively. Likewise, the validation cohort should be clinically similar to the training data to properly assess the performance of the

Figure 3 | Continued



**Clinical utility of genomic classifiers (decipher case):**  
 Decipher more appropriately directs postoperative treatment to patients most at risk of metastasis

model. If a model demonstrates superior performance compared with existing tools, it goes through to the clinical impact evaluation in prospective studies. So far, no prognostic test for predicting metastasis after radical prostatectomy has passed this step.

The Decipher platform provides true representation of the transcriptomic landscape of PCa progression in the surgical setting and captures novel transcriptomic alterations, including poorly annotated regions such as lncRNAs that cannot be detected using other platforms. These lncRNAs have been linked to biological processes involved in tumorigenesis (Prensner and Chinnaiyan, 2011) and PCa (Cheng *et al.*, 2013). It is essential to understand the molecular biology behind the Decipher biomarkers to properly understand the molecular mechanism of PCa progression. Characterising Decipher biomarker pathways is important to identify new key players that may act as therapeutic targets.

Twelve Decipher genes, including *UBE2C*, *ZWILCH*, *NUSAP1*, *MYCBP1*, *AN07*, *EPPK1*, *TNFRSF19*, *LASP1*, *THBS2*, *IQGAP3*, *CAMK2N1*

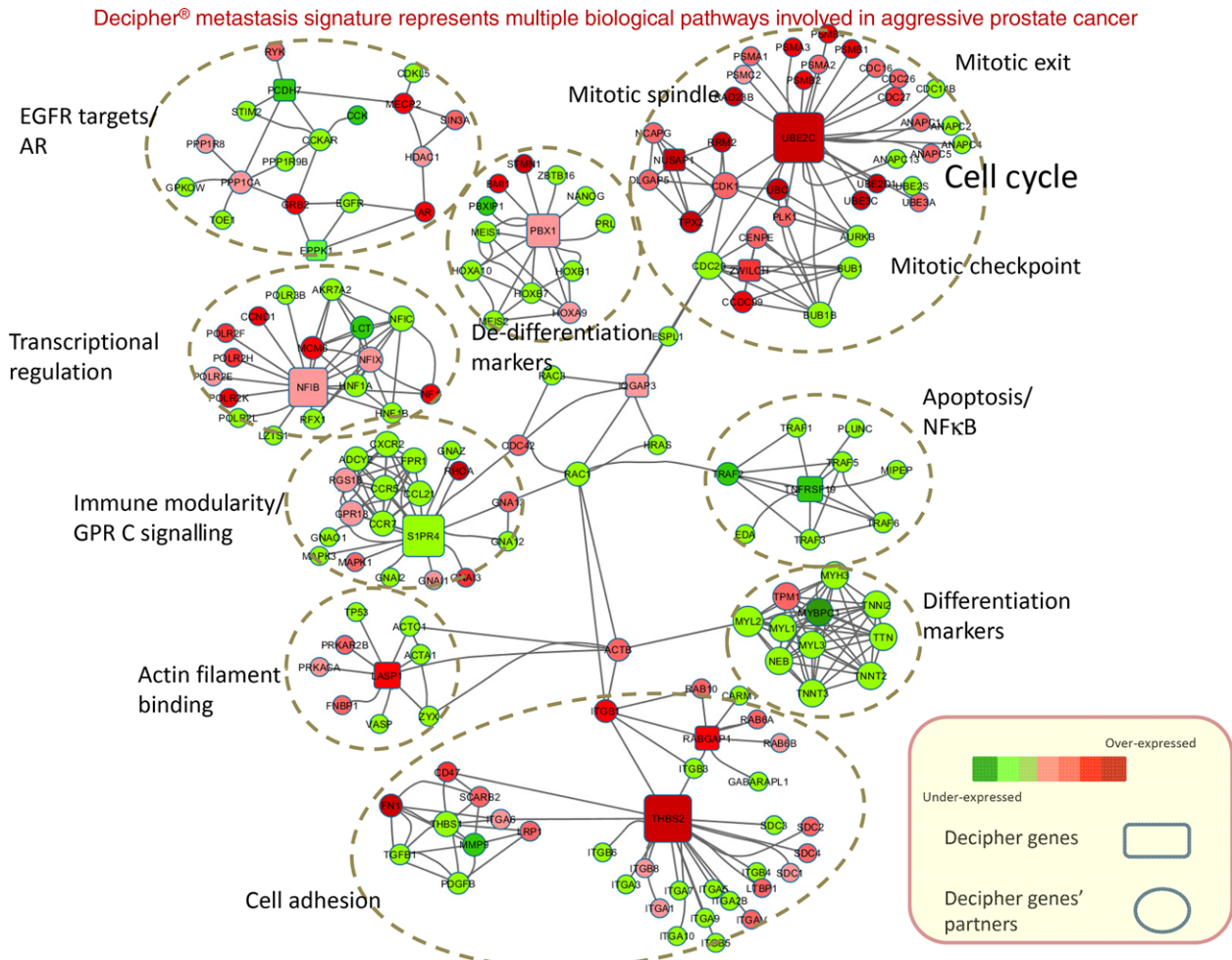
and *RABGAP1*, were in the metastatic fingerprint defined in this study. To better understand the biological role of the Decipher biomarkers in the cellular context, the protein partners of the functionally annotated Decipher biomarkers were characterised through the functional protein association network tool STRING (Franceschini *et al.*, 2013). Several Decipher biomarkers were related to PCa progression through the cell cycle and mitotic checkpoint, cell adhesion and apoptosis pathways. Ten modules capturing the function of the Decipher gene were identified (Figure 4), with the two major modules being involved in cell cycle process and mitosis and cell adhesion. Other modules are also involved key processes related to cancer, including apoptosis, cytoskeleton organisation, immune modulation, cell differentiation and transcription regulation.

Comparing Decipher signature to other PCa prognostic signature, several Decipher genes are among existing PCa prognostic signatures. For example, *UBE2C*, *NUSAP1* and *MYBPC1* are among the 86 gene Penny signature (Penney *et al.*, 2011) that



**Figure 4 |**

The systems biology landscape of the Decipher biomarkers shows modularity. The Decipher genes form 10 intertwined modules representing several pathways; mainly cell cycle, cell adhesion and cytoskeleton reorganisation. Other pathways are related to apoptosis, cell differentiation, immune modularity and inflammation.



predicts high risk cases in Gleason 7. The three genes were highly differentially expressed between Gleason  $\leq 6$  and Gleason  $\geq 8$ . *NUSAP1* is among the 31 CCP genes that constitute the Prolaris signature (Cuzick *et al.*, 2011), and *BUB1B* and *CDC20*, that are among the 31 CCP genes, are highly interacting with *ZWILCH* and *UBE2C* from Decipher suggesting the Decipher is capturing the cell cycle dysregulation aspect of tumour. Several Decipher biomarkers have been identified with links to breast cancer metastasis, bladder, and colon cancer. For example, *UBE2C* has been identified as a prognostic protein in high risk breast cancer (P syrri *et al.*,

2012), non-muscle invasive bladder cancer (Fristrup *et al.*, 2013) and node-positive breast cancer (Lous-souarn *et al.*, 2009). *LASP1* has been linked to breast cancer metastasis as a negative prognostic indicator of long-term survival of breast cancer patients (Frietsch *et al.*, 2010), ovarian cancer migration and proliferation by influencing zyxin localisation (Grunewald *et al.*, 2007) and progression and metastasis dissemination of medulloblastoma (Traenka *et al.*, 2010). Furthermore, *PBX1* is a significant prognostic marker in non-small lung carcinoma (Qiu *et al.*, 2009). Several lines of evidence have shown that *NFIB* is associated with progression of several other cancers. It serves as

an oncogene in small cell lung cancer (Dooley *et al.*, 2011) and has elevated expression in metastatic Giant cell tumour of bone (Mosakhani *et al.*, 2012) and is a potential target of oestrogen receptor-negative breast cancer as it has a role in cell proliferation (Moon *et al.*, 2011).

Characterising the association between the metastatic fingerprint and Decipher risk categories revealed its relevance to PCa risk. Additionally, patients that developed metastasis were found to fall in four subgroups (ERG<sup>+</sup>, ETS<sup>+</sup>, SPINK<sup>+</sup> and TripleNeg) suggesting a molecular heterogeneity underlying metastasis (Figure 3B). A prognostic fingerprint should offer relatively equivalent performance across subtypes to ensure reliability and robustness. Here, we found the fingerprint is independent of the PCa molecular subtypes (Figure 3B) and the Decipher PCa classifier is an effective prognostic tool of metastasis in the four subgroups supporting that Decipher is robust transcriptomic fingerprint (Figure 3C). Decipher's use as a prognostic tool has made significant impact on decision making in several published reports (Figure 3D) (Badani *et al.*, 2013), which is the ultimate goal of transforming lab developed fingerprints into clinically utilised tests.

### Challenges of translating fingerprint into prognostic tools

Many attempts have been made to develop prostate genomic tests based on transcriptomic data for PCa risk stratification, but most of them failed to be clinically adopted (Irshad *et al.*, 2013; Wu *et al.*, 2013; Bismar *et al.*, 2014). This failure can be attributed to multiple reasons. First, analytical validation in independent cohorts is essential and most developed signatures failed to be reproduced in independent cohorts. Second, the validation to predict the relevant clinical endpoint is critical; most developed gene panels are validated in different settings, different endpoints or different tissue. This could be because of the lack of the appropriate cohort for validation as only a handful of fully annotated PCa data sets are publically available. The last and most important requirement for clinical adoption is the equivalent and robust performance across a spectrum of disease subtypes that are related, but not limited, to race, age and molecular subtype.

Current emerging biomarkers aim to enable the determination of an appropriate treatment strategy

for individual patients, detect advanced disease at an earlier stage, and predict metastatic cancer and recurring disease following prostatectomy. Because of the heterogeneity of the disease, no single biomarker will be diagnostic and prognostic for every patient. The next PCa diagnostic or prognostic test should employ multiple biomarkers that could be combined with current risk assessment tools. Whether the next tools are genomic, transcriptomic, proteomic, metabolomic or a combination of all, the test should be based on genome-wide analysis to truly represent the biology underlying PCa. Robust prognostic tools better present the spectrum of tumour biology (Hanahan and Weinberg, 2011) to uncover the alterations at different levels of cancer progression. Decipher results are indicative of the dysregulation of multiple pathways and biological processes in the early stages of cancer progression, unlike other commercial tests that focus on single pathways, suggesting that Decipher provides a more complete perspective of PCa development, making it more sensitive to early molecular tumorigenic events. Currently, the Decipher test is the only prognostic test that was developed using a genome-wide unbiased approach to define the fingerprint discriminating metastatic patients. The 22 genes comprising the Decipher showed to be representing the biology underlying metastasis and impacting treatment decision.

## Materials and methods

### Patient population

A total of 1449 patient expression profiles were analysed from eight RP cohorts from Mayo Clinic (I and II) (Erho *et al.*, 2013; Karnes *et al.*, 2013), Thomas Jefferson University (TJU) (Den *et al.*, 2014), Cleveland Clinic (CCF) (Klein *et al.*, 2014b), Johns Hopkins (JHMI), Memorial Sloan Kettering (MSKCC) (Taylor *et al.*, 2010), Erasmus MC (EMC) (Boormans *et al.*, 2013) and the German National Cancer Registry (DKFZ) (Brase *et al.*, 2011) (Table S1). Five cohorts utilised RNA extracted from FFPE RP specimens and three cohorts used RNA extracted from fresh-frozen RP specimens. The Mayo Clinic I ( $n = 545$ ) cohort with 215 patients developing metastasis was used as a discovery cohort to define the metastatic fingerprint, and the remaining sets used for evaluation.

### Data generation and pre-processing

Tumour specimens were obtained from archived paraffin blocks and RNA extraction was performed using the Decipher platform as described previously (Erho *et al.*, 2013). RNA was amplified, labelled and hybridised to Human Exon 1.0 ST microarrays (Affymetrix) covering 1.4 million probesets. Annotation of features to genes was based on the alignment provided by xmapcore against the hg19 version of the human genome. Based on the

proximity of a feature relative to the genes annotated Ensembl v62, features were categorised as either coding, UTR, intronic, anti-sense and intergenic as previously described. Features overlapping with known non-coding transcripts were annotated as non-coding transcripts. More than 75% of the features on the Human Exon array cover regions annotated as non-protein coding. The SCAN algorithm (Piccolo *et al.*, 2012) was used for individual patient profile pre-processing and normalisation.

### Molecular subtyping of PCa patients

Patients in this study were classified into four molecularly distinct groups based on microarray-based classifiers, where we developed a supervised RF model that was trained to predict *ERG* rearrangement assessed by FISH. Rearrangements in other genes such as *ETV1*, *ETV4*, *ETV5* and *SPINK1* were predicted using outlier analysis method. The four subgroups were defined based on the results of the microarray-based classifiers (m-*ERG*, m-*ETS* and m-*SPINK1*) models described in (unpublished data). Patient profiles with high m-*ERG* score (m-*ERG*<sup>+</sup>) and m-*ETV1*<sup>-</sup>, m-*ETV4*<sup>-</sup>, m-*ETV5*<sup>-</sup>, m-*FLI1*<sup>-</sup> and m-*SPINK1*<sup>-</sup> were classified as *ERG*<sup>+</sup> subtype, patient profiles that were m-*ETV1*<sup>+</sup>, m-*ETV4*<sup>+</sup>, m-*ETV5*<sup>+</sup> or m-*FLI1*<sup>+</sup> and m-*ERG*<sup>-</sup> were classified as *ERG*<sup>-</sup>*ETS*<sup>+</sup> subtype, patient profiles that were m-*SPINK1*<sup>+</sup> and m-*ERG*<sup>-</sup> were classified as *ERG*<sup>-</sup>*SPINK1*<sup>+</sup> subtype, and patient profiles that are m-*ERG*<sup>-</sup>, m-*ETV1*<sup>-</sup>, m-*ETV4*<sup>-</sup>, m-*ETV5*<sup>-</sup>, m-*FLI1*<sup>-</sup> and m-*SPINK1*<sup>-</sup> were classified as the 'Triple Negative' subtype.

### Expression analysis of the PCa metastatic transcriptome

Mayo Clinic I cohort ( $n = 545$ ), a matched case control cohort, was used to develop a metastatic fingerprint. Differential expression analysis was applied to identify discriminative features between patients who developed metastasis after RP (40%) and patients who did not (60%). Two criteria were used to filter discriminative features; median fold difference (MFD) adjusted for the interquartile range across all patients (threshold 0.3), and Wilcoxon test ( $P = 0.001$ ) after MFD filtering. Features that passed these criteria formed the metastatic fingerprint.

### Author contribution

M.A. and E.D. conceptualised and designed the research; M.A., M.S., H.S., N.E. and E.D. performed the data analysis and interpretation; M.A., M.S., H.S. and E.D. wrote the article.

### Conflict of interest statement

M.A., M.S., H.S., N.E. and E.D. are employees of GenomeDx Biosciences. E.D. is the president and CSO of GenomeDx Biosciences.

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