Rospo, Giuseppe; Corti, Giorgio; Crisafulli, Giovanni; Novara, Luca; Bardelli, Alberto. Tracking colorectal cancer evolution in time and space. ANNALS OF ONCOLOGY. None pp: 0-0.
DOI: 10.1093/annonc/mdx127

Tracking colorectal cancer evolution in time and space

Giuseppe Rospo[1], Giorgio Corti[1,2], Giovanni Crisafulli[1,2],
Luca Novara[1] and Alberto Bardelli[1,2]*

[1]Candiolo Cancer Institute-FPO, IRCCS, 10060 Candiolo (TO), Italy; [2]Department of Oncology, University of Torino, SP 142 km 3.95, 10060 Candiolo (TO)

(*E-mail: alberto.bardelli@unito.it)

In the current issue of Annals of Oncology, Lote and colleagues shed light on the timelines of metastatic dissemination in a colorectal cancer patient [1]. We have long known that the process leading from a primary neoplasm to the formation of metastases involves years (often decades) [2-4]. However the precise timelines of each step have been difficult to estimate with accuracy. Lote and coworkers exploited a clever approach involving clinical annotation of metastasis seeding coupled to mathematic calculations to reconstruct the chronological evolution of a colorectal cancer.

The concept that cancer cell populations clonally evolve was proposed in 1976 by Peter Carey Nowell [5]. In his model, the tumor is the result of changes occurring in a normal cell, which displays a selective growth advantage over the general population. Over time neoplastic cells acquire additional genetic differences while maintaining similarities with their ancestors.

While the idea that tumor evolves in time is not novel, only the availability of DNA Next Generation Sequencing (NGS) technologies and the development of dedicated bioinformatic tools allowed precise assessment of cancer evolution parameters [6, 7]. Several evidences indicate that tumors develop through progressive clonal sweeps associated with acquisition of somatic mutations [2, 8]. In analogy with the structure of trees, 'trunk' mutations represent the complement of genetic alterations shared by all malignant cells within a tumor. Molecular alterations that occur early are present in every sub-clone, indicating that there must have been a single ancestral cell. All mutations that occur after the most recent appearance of a common ancestor are instead sub-clonal (branched mutations). Most of the molecular alterations acquired during evolution do not immediately affect tumor fitness (passengers

mutations), while a subset of the variations (driver mutations) provides a selective advantage [9]. Building on these premises mathematical models and bioinformatic tools were developed to track clonal evolution and reconstruct phylogenetic trees of cancers dissemination [10]. Importantly, virtually all models infer the structures of phylogenetic tree by assuming that each sub-clone maintains all the alterations of its ancestors [6, 7].

The study by Lote and colleagues adds importantly to previous work, such as the analysis of Andor and colleagues which deduced the clonal architecture of 12 different tumor types [11] using *ad hoc* bioinformatics tools such as PyClone and Expands [6, 7]. By focusing on the presence of founder (ancestor) clones across each tumor, these studies showed that the clonal architecture in different tumor types is highly heterogeneous and demonstrated univocal temporal evolution in individual tumors. In another landmark study, Murtaza and colleagues [12] used molecular profiles from tumor biopsies and circulating tumor DNA [13] to define the clonal architecture of metastatic breast cancer. Noteworthy results were also obtained examining clonal pattern at single cell resolution. In another investigation, Navin and colleagues [14] analyzed hundreds of cells in a primary tumor, thus revealing diverse sub-clonal populations and showing that tumor progression was sustained by punctuated clonal expansions. In colorectal cancer, Sottoriva and colleagues [15] proposed a “Big Bang” model for tumor growth. This model assumes that cancer grows as a single expansion, which leads over time to clonal sweeps that are not subjected to stringent selection.

Overall previous studies indicate that genomic data and mathematical models can be successfully coupled to infer phylogenetic trees, while the precise timeline of evolution of individual metastatic lesions is difficult to calculate. While genomic profiles and mathematical calculations can provide estimations for the timing of metastasis disseminations, clinical data remain essential to conform the predictions [16, 17].

How can we know exactly when an individual metastatic deposit was initiated?

One way is to exploit molecular clock methods to infer evolutionary timescales using genetic data [16]. To achieve this, Lote and colleagues cleverly exploited a rather infrequent clinical event: the formation of iatrogenic metastasis, which occasionally result from needle tract seeding [18]. They studied a 68 years old male that underwent a CT-guided lung biopsy to establish a diagnosis of metastatic colorectal carcinoma. Approximately two years later, FDG-PET-CT examination showed an unresectable left posterior chest wall mass at the site of the aforementioned lung biopsy. The day of the biopsy procedure therefore represents the time when the chest wall metastasis was originated. This information served as an *in vivo*

stopwatch to calibrate timing of evolution for the overall disease (Figure 1). To describe the methods they used, Lote and colleagues refer to 'carbon dating', a technique developed by geologists and evolutionary biologists, based on the decay of carbon-14 isotope that allows establishing the age of organic materials [19]. Translated in the clinical setting, a fixed tracking event (the iatrogenic metastasis formation) is used instead of carbon decay to calibrate the model and infer timing of the overall phylogenetic tree.

Combining this approach and bioinformatic methodologies [20] the authors initially estimated the chronology of formation of the primary tumor. This revealed that this particular colorectal cancer required over a decade to fully develop, a finding in line with previous studies that have shown similar or longer timelines for mCRC development [4]. The molecular clock information was further used to accurately determine timing of metastatic deposits in the lung, thyroid and urinary tract. This revealed that the colorectal cancer first metastasized to thyroid and lung; followed by the lung metastasis which eventually led to the chest wall lesion. Finally the disease metastasized to the left ureter. Thanks to the *stopwatch* approach the authors calculated that the primary colon cancer emerged between five and eight years prior to the clinical diagnosis and that the primary tumor metastasized to the lung and the thyroid within a year from its onset. As the authors acknowledge, some of the clinical features of this patient are rather peculiar and this cannot be considered a textbook case of colorectal cancer, accordingly it is unclear how broadly applicable are the findings.

Overall, the report by Lote and colleagues provides compelling evidence of how cross-fertilization among different disciplines (mathematics, informatics, evolutionary biology and oncology) can address challenging questions such as tracking cancer evolution in time and space. Considering the extensive analogies between evolution of species under natural selection and tumor progression over time, it is likely that concepts and methods derived from evolutionary studies will further help our understanding of how and when cancer arise and metastasize.

Acknowledgements

We thank Dr. Nabil Amirouchene for critical reading the manuscript.

Funding

This study was supported by European Community's H2020 Programme, grant agreement no. 635342-2 MoTriColor (A.B.); IMI contract n. 115749 CANCER-ID

(A.B.); Fondazione Piemontese per la Ricerca sul Cancro-ONLUS 5 per mille 2011

Ministero della Salute (A.B.); AIRC IG n. 16788 (A.B.);

Disclosures

The authors have no disclosures.

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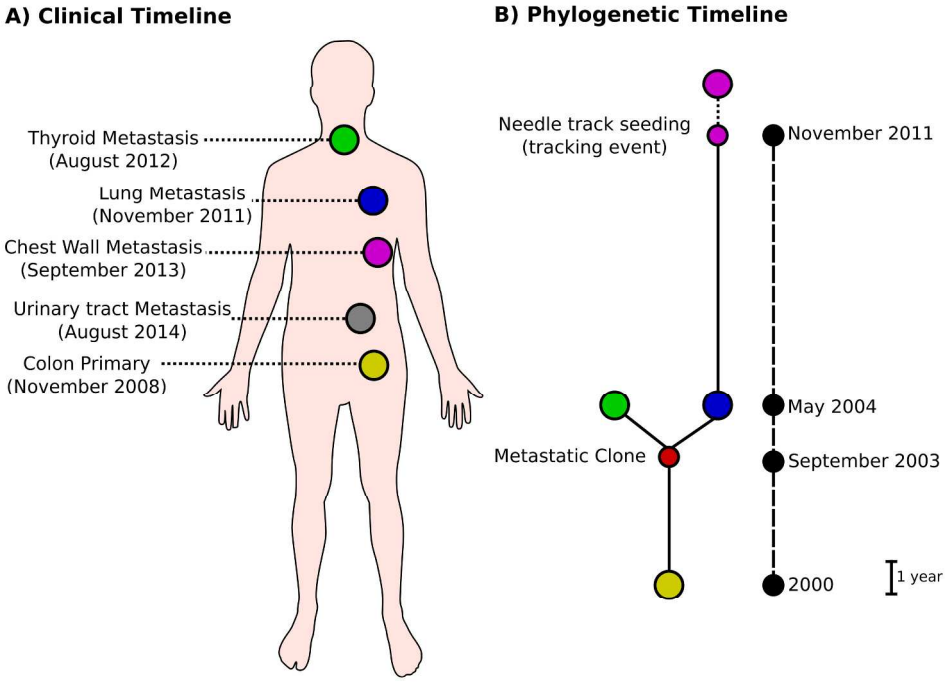
Figure 1

Spatial and temporal evolution of a colorectal cancer (A) **Clinical time-line**. In November 2008 a primary colon tumor was excised from a 68 year old Caucasian male. Three years later radiological imaging revealed a lung lesion and a new nodule in the left lobe of the thyroid. At the same time a CT-guided biopsy confirmed that the primary tumor had metastasized to the lung. In September 2013, a PET-CT demonstrated a chest wall mass caused by malignant cells seeding from the needle biopsy. In August 2014 a new urinary tract metastasis was diagnosed. (B) **Phylogenetic time-line**. The graphical representation (phylogenetic tree) shows chronological evolution of the disease. The lung biopsy was used as a tracking event to calibrate the *molecular clock* model.



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