SIMPLIFIED RESEARCH SUMMARY

Extrachromosomal DNA (ecDNA) are large, mobile, gene-containing circular DNA particles found in the nucleus of almost all highly aggressive cancerous cells. Current technologies used to detect ecDNA are compromised because ecDNA lacks many fundamental properties of DNA, so many machines confuse it for broken DNA fragments.

They inherit genes using random segregation during mitosis, leading to non-chromosomal inheritance. They have highly accessible genes and an altered gene-regulating structure due to their circular shape.

One more aspect of ecDNA is its ability to form hubs. They do this to leverage interactions as enhancers interact with promoters for faster gene transcription. ecDNAs contain only enhancers, promoters and incRNA. This suggests that the interaction of an ecDNA with other ecDNAs is what gives it power in combination.

Prevalence

By studying 14,000 patients with 39 types of tumours, we realized that 17% or 1 in 6 of all tumours contain ecDNA. A pattern was noticed that ecDNA selection is based on the tissue of origin. Shockingly, in HER2 ER-positive breast cancer, the number is remarkably high, over 26%.

- ecDNA can occur early in the conversion of regular cells to cancerous cells or later in progression (tumorigenesis), potentially hinting that it is one of the factors in tumorigenesis and disease progression.
- ecDNA has more amplifying power than oncogene amplifiers due to rapid adaptability and random high copy-number segregation.
- Amplifications occur more often in oncogenes than normal genes, suggesting ecDNA preferentially targets and distributes oncogenes in high copy numbers, promoting tumorigenesis, often beginning with a single gene alteration that produces a different protein.
- SBS signatures are more associated with ecDNA than other amplifiers, because it is easier for open chromatin to mutate than packed chromatin.

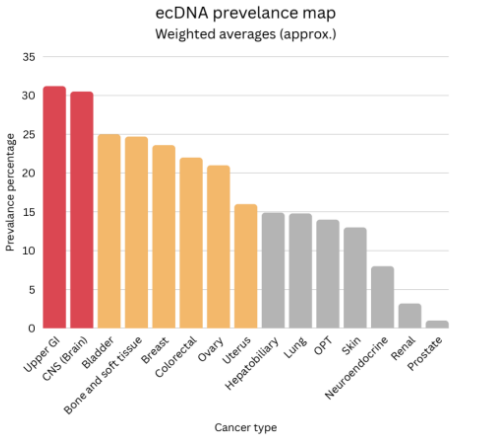

Figure 1: ecDNA prevalence map.

ecDNA and the Immune System

Studies suggest that ecDNA-driven cancers respond lesser to immune checkpoints because they have a pattern of transcribing that leads to immune suppression. We still do not know exactly why .

- 34% of ecDNA-containing tumours had immunomodulatory genes amplified, and most were co-amplified with oncogenes.
- 41.5% of the tumours with immunomodulatory genes amplified did not have oncogenes, suggesting that these immunomodulatory gene amplifications have their own role in tumorigenesis.
- ecDNA also amplifies immunomodulatory genes and immune effector processes, and these immunomodulatory genes were involved in negative regulation, meaning they helped facilitate more cancer growth.
- ecDNA tumours with both oncogene and immunomodulator amplification had higher depletion of killer T cells compared to only oncogene-amplified ecDNA.

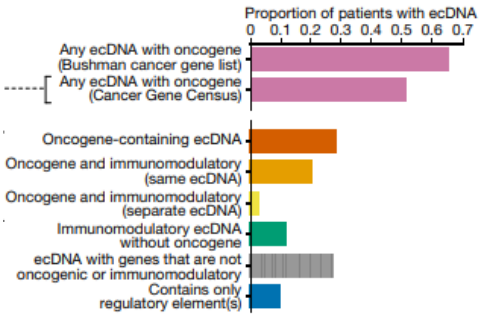

Figure 2: Proportion of patients with ecDNA

Regulatory vs Coding ecDNA

Compared to coding ecDNA, regulatory ecDNA has a higher number of promoters and enhancers, which means a higher transcription and execution rate. Enhancer-only ecDNAs were frequently co-amplified with ecDNA containing oncogenes on separate ecDNA and tended to be smaller with a lower copy number.

Mutations and Genomic Instability

The relationship between ecDNA and specific factors is largely unknown for most cancers, though TP53 mutations are strongly associated with ecDNA and account for more than 50% of all cancer mutations.

- ecDNA is more strongly associated with TP53 mutations in endometrial, renal and ER+ breast cancer.
- Whole genome duplications, wGII and structural variant burden were all associated with ecDNA.
- Cancers with ecDNA showed more mutations compared to those with other amplifiers due to highly accessible chromatin. Except hypermutation, which is negatively correlated with ecDNA in colorectal and endometrial cancer.
- Mutations present on all ecDNA copies were present before ecDNA formation. Mutations not present across copies occurred after formation.
- Tobacco, UV light and age-related mutations are the primary drivers before ecDNA formation, accumulating more at the time of ecDNA conception. DNA repair deficiencies, on the other hand, occur after ecDNA formation.

Conclusion

- ecDNA detection is directly related to prior treatment.
- The overall survival curve is steeper for ecDNA compared to any other amplifiers, as it is associated with lower OS.
- ecDNA is significantly more present in metastatic cancer, meaning it is directly correlated with cancer progression and metastasis.
- We need better and more robust sequencing techniques to account for other factors.

Our research suggests that different cancers lead to different ecDNA structures. This explains why it is fundamentally hard to find a universal cure to cancer.