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# METASTASIS AND CIRCADIAN RHYTHM


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Research in our laboratory, supported by a UKRI Future Leaders Fellowship, is dedicated to exploring the timing of metastasis. We aim to determine when metastasis occurs in various cancers, elucidate the molecular mechanisms through which circadian rhythm regulates the generation and metastatic spread of CTCs and identify gene vulnerabilities that could serve as targets for the development of novel anti-metastatic chronotherapeutics.

Metastasis is the leading cause of all cancer related deaths, accounting for nearly 12 fatalities every minute. Despite recent advances in early cancer detection, 50% of patients are either diagnosed with metastatic disease upon presentation or develop metastases after initial diagnosis with localised disease. The lack of effective anti-metastatic therapies poses a challenge in clinical practice, highlighting an urgent, unmet need that must be addressed promptly.

In our lab, we delve into metastasis through the study of Circulating Tumour Cells (CTCs). These are cancer cells that break away from the primary or metastatic tumour and through the blood circulation, they colonise distant organs thereby seeding new metastatic lesions. Thus, targeting CTCs holds immense potential for impeding metastasis, necessitating an in depth understanding of their biology to develop novel therapeutic strategies.

The rarity of CTCs in the bloodstream (average number of 5-10 CTCs per 7.5 ml of peripheral blood) poses a challenge in their detection and isolation. Leveraging our lab's expertise, we capture viable CTCs and scrutinise their expression profile and biological properties. We analyse samples from cancer patients and a range of cancer mouse models available at the CRUK Scotland Institute and we employ a combination of state-of-the-art microfluidics and robotic technologies, along with single-cell analysis methods, next generation sequencing, genetic engineering, CRISPR screens and imaging techniques to unravel the biology of CTCs and understand metastasis.

Recently, we demonstrated that CTCs disseminate during sleep, unveiling a key role of the circadian rhythm in metastasis. We analysed blood samples from hospitalised women with progressive breast cancer collected during the active (10:00am) and rest (4:00am) phases of the same day and we found a striking prevalence of CTCs during the nighttime. We also used different mouse models of breast cancer and examined spontaneous CTC generation over time. Similar to patients' data, we detected more CTCs during the mouse rest phase (corresponding to daylight time due to inverted circadian rhythm of rodents compared to humans) (Figure 1b-d). Additionally, we characterised a unique gene expression profile in CTCs induced by circadian rhythm regulated hormones during the rest phase, enhancing the metastatic potential of CTCs (Figure 2).

Building upon these findings, we delve deeper into the intriguing link between the circadian rhythm and metastasis, aiming to leverage the acquired knowledge to develop time-tailored personalised prognostic approaches along with effective anti-metastatic therapies adapted to patients' circadian clocks. Specifically, our research is structured around the three following interconnected questions:

1. Why is metastasis formed at a specific time of the day?
2. How can we block metastasis?
3. Will therapies be more effective if we administer them at specific times of the day?

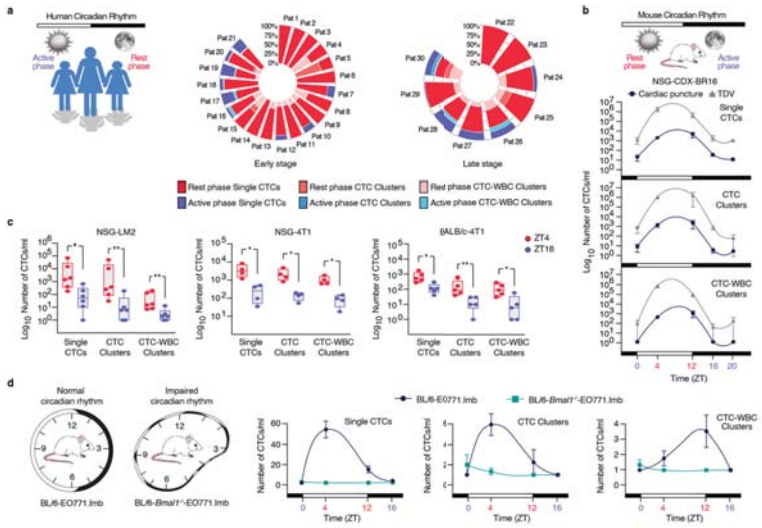


**Figure 1. CTCs intravasate during the rest phase of the circadian rhythm. (A)** Graphical representation of the human circadian rhythm. The white and black bars represent environmental light (active period) and dark conditions (rest period), respectively (left). The radial histograms show the percent of CTCs isolated during the rest or active phase in breast cancer patients. **(B)** Graphical representation of the mouse circadian rhythm. The white and black bars represent environmental light (rest period) and dark conditions (active period), respectively (top). Time kinetic analysis showing CTC counts in the NSG-CDX-BR16 breast cancer mouse model, from blood collected via cardiac puncture or tumour draining vessel (TDV) over a 24-hour time period. **(C)** Box plots showing the distribution of the number of CTCs collected at ZT4 or ZT16 in immunocompromised NSG-LM2 and NSG-4T1 or immunocompetent BALB/c-4T1 breast cancer mouse models. **(D)** Graphical representation of physiological (BL/6-EO771.lmb mice) versus impaired circadian rhythm (BL/6-Bmal1-/--EO771.lmb mice) (left). Graphs showing time kinetic analysis of CTC counts in the BL/6-EO771.lmb and BL/6-Bmal1$^{-/-}$-EO771.lmb mice. Data in panel "b" and "d" are presented as mean ± s.e.m.; for panels "c" center lines in the box represent the median; box limits represent first and third quartile; extremes of the whisker lines represent the minimum and maximum observed values. * P < 0.05, ** P < 0.01 by two-sided Mann-Whitney test. n represents the number of biologically independent mice.

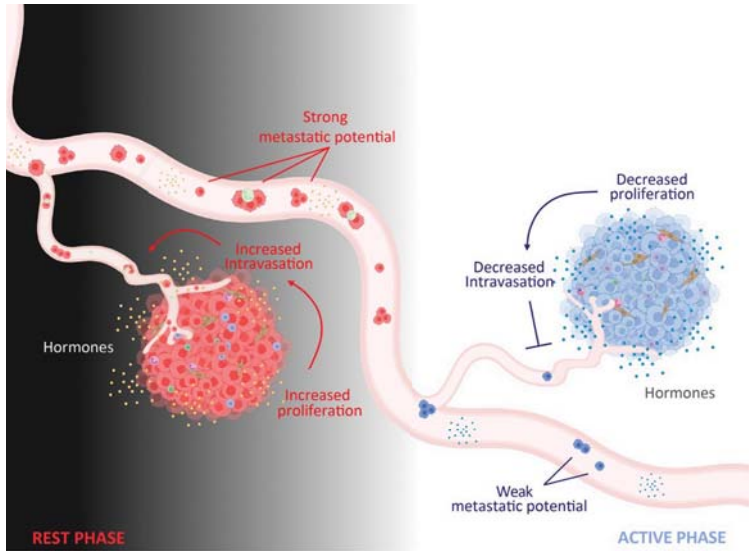


**Figure 2. Proposed mechanism for the regulation of metastasis by the circadian rhythm.**