Our Group uses cryo-electron microscopy (cryoEM) to determine the 3D structure of large macromolecular complexes of relevance in cancer at high resolution. Structural information, in combination with molecular and cell biology and biochemistry, is then used to propose how these molecules work and increase our understanding of the molecular basis of cancer. Most of our efforts are currently focused on 2 major areas of research: the study of chaperones essential for the activation of several macromolecular complexes relevant in cancer such as those formed by the mTOR kinase, and the study of complexes implicated in the repair of DNA double-strand breaks. In addition, and in collaboration with other groups, we are studying the structure and mechanisms of several amino acid transporters.

“We have characterised the structure and the molecular mechanisms of 2 protein complexes (TELO2-TTI1-TTI2 and LAT2/CD98hc) and 1 long non-coding RNA, considered important players in cancer.”
Structural biology highlights

**Structure of the TEL02-TTI1-TTI2 complex and its role in mTOR activation**

As part of a collaboration with Laurence H Pearl and Chrisostomos Prodromou at the Genome Damage and Stability Centre, University of Sussex, UK, we helped to determine the structure of the TEL02-TTI1-TTI2 complex using cryoEM. This complex is essential for the maturation and activation of mTOR, a serine/threonine protein kinase that regulates several essential processes such as cell growth, cell proliferation, cell motility, autophagy, and protein synthesis. The mTOR signalling pathway is often activated in tumours, and the pathway is being studied intensively in the search for anti-cancer therapies. The structure of the TEL02-TTI1-TTI2 complex that we helped to resolve, together with biochemical experiments, revealed some of the mechanisms involved in the activation of mTOR by chaperones.

**Long non-coding RNAs in DNA double-strand breaks in hepatocellular carcinoma**

Long non-coding RNAs (lncRNA) are now considered essential players in cancer but the mechanisms are poorly understood. As part of a consortium involving several institutions in Europe and the USA, and directed by Puri Fortes at the Centre for Applied Medical Research (CIMA), University of Navarra, in Pamplona (Spain), we contributed to studying the mechanisms of NIHCOLE, a novel lncRNA induced in hepatocellular carcinoma (HCC), whose expression is associated with poor prognosis and survival. In a close partnership between our group at CNIO and the group of Fernando Moreno-Herrero at the CNB-CSIC in Madrid, and with funding from the local Government of Madrid, we used single-molecule imaging methods (AFM and electron microscopy) to characterise the structure of this lncRNA. These images show that NIHCOLE functions as a scaffold promoting the assembly of large multimeric complexes of proteins involved in the repair of DNA double-strand breaks.

**Structure of heteromeric transporters of neutral amino acids**

Amino acids play a central role in cellular metabolism. The transfer of amino acids across the plasma membrane is performed by proteins that bind and transport these molecules from the extracellular medium into the cell, and vice versa. Heteromeric Amino Acid Transporters (HATS) are a family of amino acid transporters that harmonise amino acid concentrations at each side of the plasma membrane, and they play a significant role in cancer and several inherited diseases. Several loss-of-function mutations in human LAT2/CD998hc are associated with age-related hearing loss and cataracts, and its overexpression in pancreatic cancer cells sustains glutamine-dependent mTOR activation to promote glycolysis and chemoresistance. LAT1/CD98hc is also linked to cancer and autism.

Each member of the HAT family displays a preference for transporting a certain set of amino acids. This specialisation explains the function of each HAT family member in certain physiological processes and diseases. The molecular mechanisms explaining why each family member shows exquisite preference for transporting some amino acids but not others had been mostly unknown. We determined the structure of one such member of the HAT family, LAT2/CD998hc, using cryoEM. This structural information, together with molecular dynamics and mutational and functional studies, enabled us to specify a few residues present in the substrate-binding pocket that contribute to determining substrate preference.

**Long non-coding RNAs that counteract the mTOR signalling pathway in hepatic cancer**

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