**SOFIA FRANCIA**

Research Activities

**Use of DNA-damage induced RNA (DDRNA) mimics to enforce DNA damage response activation in the absence of DNA lesions.**

Our group and others recently published that the safeguard of DNA integrity depends on a novel class of short non-coding RNAs called DNA damage response RNAs (DDRNAs) that act at DNA lesions to promote a DNA damage response (DDR) (Francia S. et al. Nature 2012; Wei Wei et al Cell 2012; Michalick et al NAR 2012). The role of DDRNAs in DDR was established in a conveniently engineered cellular system, which allows the controlled induction of a single DNA lesion in a defined and traceable locus. With this cell system, we showed that chemically synthesized DDRNAs homologous in sequence to DNA flanking the damage site, control DDR foci formation in a dose dependent manner. More recently, we obtained unpublished data demonstrating that DDRNAs act at sites of DNA damage by pairing to nascent transcripts emerging from DNA Double strand breaks (DSBs) (Michelini F. et al submitted) and promote the accumulation of DDR mediators to site of DNA damage but are dispensable for the initial recognition of DNA lesion (Francia S. et al. Submitted).

Since the tethering of key DDR factors to an genetic locus is sufficient to trigger full DDR signaling even in the absence of physical DNA lesions (Soutoglou E., Science 2008), we hypothesized that exogenously introduced DDRNAs may be sufficient to stimulate DDR factors recruitment and DDR activation in target cells. Indeed, preliminary data from our group show that transfection of synthetic DDRNAs induces DDR activation on a complementary genomic locus, and this occurs in a relevant fraction of cells even in the absence of a preexisting DNA lesion. Prompted by these preliminary results, we want to test if the formation of a DDR focus induced by DDRNAs transfection in the absence of DNA lesions, also leads to the activation of a DNA damage cell-cycle checkpoint specifically in cells bearing target sequences, with the consequence of reducing their proliferation. This could give a selective proliferative disadvantage to cells harboring a specific genetic mutation in a mixed population, with potential relevant implications. To test this intriguing hypothesis, we will use a mixed population of target and parental cell lines, which only differ for the presence of an engineered target genetic locus and we will transfect it with sequence-specific DDRNAs, or sequence-unrelated oligos as control. Upon multiple rounds of transfection, we will measure the clonal expansion of the two cell lines in time. Measurements will be performed by Q-PCR on genomic DNA, using cell type specific primers. Results obtained will be confirmed in the different cellular systems (NIH3T3, HCT 116 and H1T1080) and, if the hypothesis holds true in in vitro experiments, we aim to confirm it by an in vivo approach, targeting with DDRNAS conveniently chosen cancer cell lines inside tumors formed in nude mice.

In summary, with this project we will test the possibility that sequence-specific, synthetic DDRNAs may be used to induce a selective proliferative disadvantage to a specific target cell population bearing genetic alterations such as mutations or viral integrations, even in the absence of physical DNA damage.
**A novel role for DICER in breast cancer prevention**

Cancer is a disease of the genome, which become unstable. Genome integrity is continuously challenged by DNA lesions, so a major cause of genome instability leading to cancer is the lack of efficient repair. The cascade of events that starts with the detection of DNA lesions and proceeds with their signalling and repair is known as DNA damage response (DDR). Among different kinds of lesions, DNA double-strand breaks (DBS) are very risky and frequently need to be repaired in a more accurate way by the homologous recombination repair (HRR) pathway that uses the intact chromatid as a template. Breast cancer is the most common malignancy in women worldwide and its incidence is increasing in developing countries. Mutations of BRCA1 and BRCA2 are responsible for about 50% of hereditary breast cancers, and account for 8% of all breast cancers (Banerji et al., 2012), (Carraro et al., 2013). Moreover, BRCA1 expression is frequently reduced in sporadic cancers, suggesting a much wider role in mammary carcinogenesis and patients with BRCA mutations develop also ovarian cancer (Einbeigi et al., 2010), (Liu et al., 2007). Both BRCA1 and BRCA2 are required for maintaining genome integrity being involved in DNA repair and cell cycle checkpoint control, therefore the complete loss of function of either protein leads to a dramatic increase in genomic instability (O’Donovan and Livingston, 2010). Recently, Sofia Francia identified a novel class of non-coding RNA (ncRNA) involved in local DDR activation, DDRNAs (Francia et al., 2012), which are processed by the RNase type III enzyme DICER, historically characterized in the pathway of the RNA interference. DDRNAs have the sequence of the damaged DNA and control DDR activation and cellular senescence maintenance (Francia et al., 2012). More recently, others groups reported an involvement of sequence-specific DICER dependent ncRNA, so DDRNAs, in HRR (Gao et al., 2014), (Wei et al., 2012). Therefore, it is becoming apparent that DNA DBS induce the formation of ncRNA that are essential for repair by HR (d’Adda di Fagagna, 2014), (Gao et al., 2014), (Fong et al., 2013). With the proposed project, I aim to test the hypothesis that DICER and its RNA products DDRNA by playing and important function in HRR, prevents breast cancer origination. Indeed, emerging evidence shows that inactivating mutations in DICER gene is associated with increased cellular transformation and tumorigenesis (Zhang et al., 2013), (Heravi-Moussavi et al., 2012), (Hill et al., 2009), (Merritt et al., 2008), (Kumar et al., 2007) and this is particularly relevant in breast cancer where DICER expression is frequently down-regulated (Khoshnaw et al., 2012), being lower in more advance tumour stages (Grelier et al., 2009) and more frequent in triple-negative breast cancer (TNBC) (Avery-Kiejda et al., 2014). Moreover, a poor clinical outcomes was observed among patients with validated low DICER expression (Noh et al., 2011). Up to now, this observations has been interpreted in the light of the canonical RNAi functions. However, the discovery of DDRNAs suggests an additional interpretation of this fact pointing to the requirement of these genes for DDRNAs biogenesis, DDR-enforcement and DNA repair by HRR. Therefore, I plan to test the role of DICER and DDRNAs biogenesis in genome instability, cellular transformation and breast cancer development. The study will be initially performed in cultured cells by inactivating BRCA1 or BRCA2 or DICER (or a combination of these genes) by siRNA technology, to be able to investigate with different approaches how DICER affect DNA repair in proliferating BRCA1 and/or BRCA2 negative cells, but later I plan to validate this working model in specimen from cancer patients, in collaboration of Francesco Nicassio at
IIT@SEMM in Milan. Importantly, since breast cancer is frequently treated with the PARP-inhibitors Olaparib, a drug which selectively kills breast cancer cells by inhibiting other repair pathway that compensate for an inactive HRR, we will test potential synergistic or antagonistic effect of DICER inactivation with PARP inhibition in the treatment of BRCA-deficient cells. Next, we plan to obtain primary cell lines from human patients carrying BRCA-mutation or TNBC (that retain BRCA-but downregulate DICER) and we will use them to validate our results with Olaparib. Finally, since we observed that DICER inactivation, allow senescent cells to escape the DDR dependent cell cycle arrest, we plan to investigate how senescence and the associated secretory phenotype (SASP) is affected by DICER inactivation in breast cancer cells. To do so we will correlate the level of DICER expression in different stages of cancer progression with the positivity to beta-galactosidase in specimen of breast cancer and then we will evaluate the production of SASP associated interleukins, by Q-RT-PCR in RNA samples from the same tumours. This will help us to understand how senescence induction and SASP are affected by loss of DICER expression during breast cancer progression. Next, we will corroborate our conclusion by analysing published dataset of gene expression in breast cancer samples. With this project we believe we can identify a novel mechanisms affecting genome instability, cell transformation and tumour progression during breast cancer development. Prospectively, if the hypotheses tested hold true, this will open new possibilities for the comprehension, the diagnosis and the treatment of breast cancer patients.