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COMMUNICATION

Response to communication of Paola Romagnani and Giuseppe Remuzzi

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We thank P Romagnani and G Remuzzi for their comment (Romagnani and Remuzzi, 2014) on our paper (Bombelli et al., 2013). The goal of our paper was to obtain a cell population enriched with adult renal stem cells exploiting the self-renewal characteristic, through the formation of clonal nephrospheres, adapting the sphere-forming assay to fresh human renal tissue. We avoided the use of markers because they are known to be promiscuous and expressed not only by stem cells but also by progenitors and differentiated cells (Sagrinati et al., 2006; Pece et al., 2010). With our approach, we obtained cells with properties of stem cells, as has been previously shown by others that have provided reliable evidence in obtaining stem cells from different human tissues (Reynolds and Weiss, 1992; Dontu et al., 2003).

After staining with fluorescent PKH26 dye (Pece et al., 2010), the brightest renal cells (PKHhigh) in the nephrospheres were the only ones able to generate new filial nephrospheres. Thus our PKHhigh cells showed self-renewal capacity, with a calculated Sphere Forming Efficiency (SFE) of about 72%, and were also able to differentiate into epithelial, podocytic, and endothelial lineages in vitro. The SFE of fresh human renal tissue cells was 0.69% (see Table S2 in our paper). Therefore, our PKHhigh cells represent a cell population enriched with cells that show characteristics of stem cells. The previous use of CD133 and CD24 to indicate human renal progenitor cells (Sagrinati et al., 2006; Bussolati et al., 2005; Lazzeri et al., 2007; Ronconi et al., 2009; Sallustio et al., 2010; Lindgren et al., 2011; Angelotti et al., 2012; Bussolati et al., 2012) led us to evaluate their expression in PKHhigh cells, showing that the distribution of these markers was heterogeneous.

Romagnani and Remuzzi say that "CD133+/CD24− cells do not exist in vivo," and our results can be "related to technical differences and/or culture manipulation".

In recent years, with the availability of validated conjugated antibodies, direct staining is widely used, even when coexpression studies are needed (Sallustio et al., 2010; Buzhor et al., 2011; Bussolati et al., 2012; Tanqri et al., 2013). We obtained the same results on nephrosphere cells using either FITC or PE conjugated CD24 antibodies. Moreover, in our PKHhigh cells the absence or the not detectable low expression of CD24 characterizes a cellular subset that is different from the one in which CD24 is well expressed. This difference is supported also by the different stem cell capacities. In fact, CD133+/CD24−/PKHhigh cells generated new filial nephrospheres and differentiated toward the epithelial, podocytic, and endothelial lineages; CD133+/CD24+/PKHhigh cells generated new filial nephrospheres as well, but differentiated toward epithelium and podocytes only, like the cells described by the Romagnani group (Sagrinati et al., 2006; Ronconi et al., 2009); the CD133−/CD24−/PKHhigh cells were not able to form nephrospheres. Otherwise, in Lazzeri et al. (2007) (Fig. 1A), in Ronconi et al. (2009) (Fig. 2A,B), and in Angelotti et al. (2012) (Fig. 1A) the existence of CD133+/CD24− cells cannot be excluded. By FACS analysis, after indirect staining of freshly isolated adult renal cells, it is possible to notice that a low percentage of CD133+/CD24− events have been detected. In addition, Bussolati et al. (2012) (Fig. 3A) show a consistent amount of CD133+/CD24− events by FACS analysis on CD133+ cultured renal cells after direct staining.

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Of course, we agree that it is important to establish the existence of the putative stem cell population in fresh kidney tissue. However, we found that CD133+/CD24− and CD133+/CD24+ cells were also present in our cell populations that were unable to generate new filial nephrospheres and therefore not capable of self-renewal (PKHlow, PKHneg cells, and differentiated primary cell cultures). This means that the expression of these markers is not related "per se" to the self-renewal and multipotent capacities. Stem cell properties are dispersed among the cells also carrying these markers, but the specific stem cell phenotype is probably something hidden under the PKHhigh status and a transcriptomic analysis of the self-renewing PKHhigh cells may reveal it. We are convinced that the specific signature, identified on a homogeneous cell population with self-renewal and multipotent capacity, has to be obtained to precisely identify stem cells on fresh tissue, overcoming the problems of promiscuity shown by the markers currently available. We are currently trying to obtain this signature on our self-renewing PKHhigh cells and then we will go back to the tissue to identify and localize stem cells in the context of a possible renal stem cell niche on both physiological and pathological fresh renal tissues.

Moreover, it has been reported that CD133+ cells are not detectable in human fetal renal tissue, while the cells isolated from the same tissue and FACS-analyzed show a detectable CD133 expression, not always co-expressed with CD24 (Metsuyanim et al., 2009, Fig. 4C). These data support the described possible discrepancy between FACS analysis and detection of some markers directly on tissue (Stacchini et al., 2012; Dong et al., 2007) and make stronger our choice to obtain first the signature of renal stem cells and then look for their presence on fresh tissue.

Buzhor et al. (2011) state that "aggregation into hKEpC spheroids had occurred and that cells were not entirely clonally derived." Therefore, their spheroids are not easy to compare with our clonal nephrospheres. In any case, we do not exclude that culture manipulation may determine modulation of cells with self-renewal capacity, and this can be an interesting observation that could open possible translational applications.

In conclusion, we do not think that the precise phenotype of adult renal stem cells has yet been determined, and we hope to contribute to this by using our functional approach.

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